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A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

Abstract:

Abstract of WO9805787

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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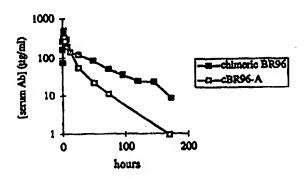
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Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

(57) Abstract

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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5 A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods for inhibiting or reducing immunoglobulininduced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of
using unmodified antibodies or recombinant binding proteins for in vivo use, the
invention provides the use of modified antibodies or recombinant binding proteins
which have been structurally altered in the constant domain so that upon
administration immunoglobulin-induced toxicity is reduced or inhibited.

BACKGROUND OF THE INVENTION

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Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al., Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH₂ domain, the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

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Others have generated a CH₂-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)). Their findings provide that the CH₂-deleted antibody was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH₂-deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity, increased target specificity, and rapid clearance from circulation (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990).

Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent interactions. Each chain contains a variable region (V) and a constant region (C). The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH₁) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,

depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH₂) is adjacent to the hinge region. CH₂ contains sequences important for effector functions of the antibody, such as the sequences responsible for complement fixation, and Fc receptor binding The third constant region domain (CH₃) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

25 SUMMARY OF THE INVENTION

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The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

Structural alteration of the constant region may be effected in a number of ways as long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH₂ domain is deleted. In another embodiment, only that portion of the CH₂ domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH₂ domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

Alternatively, structural alteration is effected by single or multiple mutations in the CH₂ domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

Further alternatively, structural alteration can be effected by isotype switching resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a line graph showing plasma clearance in high LeY expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

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Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the

human (h)BR96-light chain (SEQ ID NO. 13). 10

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the

CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

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Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH2 deletion (PCT Application No. 95/305444, published March 6, 1996)).

Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96. 20

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Ley (closed diamond), (2) hBR96-2A to Ley (96:0006A2 R/A)(closed

square), (3) hBR96-2A to Ley (96:0006B R/A)(closed triangle), and BR96-Dox to

 $Le^{y}(X)$.

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Ley (closed diamond), (2) chiBR96 to Ley (closed square), (3) cBR96-A to Ley (96:0003 R/A)(closed triangle), and cBR96-Dox to Ley (X).

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Figures 9a-c are schematic diagrams showing the steps for deleting a CH₂ domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH₂ domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.

Figure 13 is a schematic diagram showing the construction of pD17-hJm14-

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in Figure 5, chimeric BR96 having the CH₂ deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole chiBR96 and deleted CH₂ chiBR96 on Le^y.

20 Figure 16 is a description of the seven structural alterations.

dCH2.H1.

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Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.

Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudonomas aeruginosa* flagella type b mAb, negative control.

Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-Pseudonomas aeruginosa flagella type b monoclonal antibody (mAb), negative control.

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Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

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Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

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Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of he 1.4 kpb IgG heavy chain region showing the hinge CH₂ and CH₃ domains as boxed regions. Site-specific mutations to be introduced into CH₂ positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR produces as is shown in the four-way recombination of RsA2, A1B1, B1Ra with vector.

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Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH₂ domain.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

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The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by symptoms other than those described above.

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated domain.

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Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH₂ domain of the constant region. In this instance, deletion of the entire CH₂ domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of the CH, domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH₂ domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity. For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) Annu. Rev. Immunol. 8:303-333; T. Honjo et al. (1979) Cell 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

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As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 METHODS OF THE PRESENT INVENTION

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The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

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In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le^x. In another embodiment, the immunoglobulin recognizes and binds Le^x. In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10036. In yet another embodiment, the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10460.

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In accordance with the practice of the invention, the immunoglobulin can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC binds. Also, in accordance with the practice of the invention, the immunoglobulin can be an anti-idiotypic antibody.

As required by the invention, at least a portion of the constant region of the immunoglobulin molecule is structurally altered. Structural alteration can be effected by a number of means. In one embodiment, the entire constant region, i.e., CH₁, CH₂, and CH₃ domains, can be deleted.

In another embodiment, only the CH₂ domain is deleted from the immunoglobulin molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH₂ deletion may result in a molecule unable to bind the Fc receptor or a complement component.

In another embodiment, only that portion of the CH₂ domain which binds the complement component Clq is deleted. In yet another embodiment, mutations in specific portions of the CH₂ domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

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Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a ⁵¹Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

In another embodiment of the invention, the method comprises administering to the subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH₂ domain so that the altered molecule no longer binds the Fc receptor or a complement component.

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one embodiment, the antibody recognizes and binds Le^y. In another embodiment, the antibody recognizes and binds to Le^x.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

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Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates symptoms associated with the disease.

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In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH₂ domain of the constant region of the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

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Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as ¹³¹I; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

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Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH₂ domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include,

but are not limited to, liquid solutions or suspensions, tablets, pills, powders,
suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable
or infusible solutions. The preferred form depends upon the mode of administration
and the therapeutic application.

25 The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

Additionally, in accordance with the invention, the lipid carrier can be a liposome.

As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

The most effective mode of administration and dosage regimen for the compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m² of surface area is described by Freireich, E.J., et al. Cancer Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

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administered daily or proportionally reduced depending on the specific therapeutic situation).

THE MOLECULES OF THE INVENTION

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The present invention provides structurally altered BR96 or BR96 Ig fusion proteins. Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 lg fusion proteins have been made.

- In one embodiment, designated cBR96-A, the entire CH₂ domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.
- In another embodiment, designated hBR96-2A, the entire CH₂ domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.
- In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; and the proline residue located at position 331 is mutated to alanine.

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In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

In another embodiment, designated hBR96-2H, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

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Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

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Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons 10 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA (cDNA), or ribonucleic acid (RNA).

IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or lg fusion proteins) may be constructed using a wide variety of chemotherapeutic agents such as folic acid and 10 anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including 15 doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-20 27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

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Suitable therapeutic agents for use in making the immunoconjugate includes <u>Pseudomonas</u> exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic agent.

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

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Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

Alkylating agents include mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapuetic agent aminopterin has a correlative improved analog namely methotrexate.

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Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is cyclophosphamide.

METHODS FOR MAKING MOLECULES OF THE INVENTION

There are multiple approaches to making site specific mutations in the CH₂ domain of an immunoglobulin molecule. One approach entails PCR amplification of the CH₂ domain with the mutations followed by homologous recombination of the mutated CH₂ into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the f1 origin of replication. This gives the vector the properties of a phagemid and site-directed mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

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In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

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EXAMPLE 1

The following standard ELISA protocol was used.

20 Materials: Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')₂

Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research),
Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le^y-HSA
(Alberta Research Council).

Methods: Dilute primary antibody or antigen to 1.0 μ g/ml in 0.05M Carb/Bicarb buffer. Add 100 μ l of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

Block plates by flicking them and blotting on paper towels. Add 200µl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

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Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

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For conjugation add 100 μ l/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H₂SO₄ 100 µl/well. Read plate at 450/630nm in EIA plate reader.

EXAMPLE 2

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Construction of CH2 deleted BR96 molecules

Strategy for Deleting CH₂ Domains: To construct CH₂ deleted BR96 molecules, the hinge, CH₂ and CH₃ domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH₃ domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pNγ1.14) molecule lacking the CH₂ domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH₃ domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

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A sewing PCR strategy was used for the construction of CH₂ deleted human IgG1 (pNy1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH₁ domain was amplified as a 580 bp fragment with a sense oligonucleotide

(5' TGG CAC CGA AAG CTT TCT GGG GCA GGC CAG GCC TGA 3') (primer A) and an antisense oligonucleotide (5' TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B) from a linearized human IgG1 constant region vector (pNγ1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra-III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH_I domain.

The CH₃ domain was then partially amplified (to the Xba-I site) with a sense primer (5' GTC GAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA

25 TGG ACA GAG GCC GGC T 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC TCT AGA TGG 3') (primer D) from a linearized human IgG1 constant region vector (pNγ1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-1 site (in bold) within the CH₃ domain.

The CH₁ and CH₃ partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and reannealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH₁ - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH₃ partial - Xba-1.

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The combined PCR fragment, with the CH₁ and partial CH₃ domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

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To transfer the CH_1 and partial CH_3 into a mammalian expression vector, both the pEMBL18 and pN γ 1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pN γ 1.7 vector. The new construct, with CH_1 and a full CH_3 domain, was designated the pN γ 1.10 vector.

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The hinge fragment was amplified from a Hind-III digested pNy1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH₁ and CH₃ domains of the pNy1.10 construct. The sense oligonucleotide (5' ACC ATG GTC GAC CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT CAC GTG GTG TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplication of the hinge fragment (250 bp).

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pNγ1.10 with the CH₂ and CH₃ domains were digested with Sal-1 and Dra-III. The digested hinge fragment was cloned into the Sal-1 and Dra-III linearized sites on the pNγ1.10 vector. The new construct, now carrying the CH₁, hinge and CH₃ domains, was designated pNγ1.11.

To make the final CH₂ deleted human IgG1 construct, both the pNγ1.11 construct and pNγ1.11 vector were digested with BamH1 and HindIII. A fragment containing the CH₁, hinge and CH₃ domains was cloned into the linearized pNγ1.11 vector. The new constant region IgG1 construct lacks the CH₂ domain and is designated pNγ1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH₂ and CH₃ domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH₁ and hinge and the 3' end is located inside the CH₃ intron of the BR96 IgG1 molecule. The hinge, CH₂ and CH₃ domains (1.368 kb fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

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The CH₂ deleted BR96 IgG1 was then constructed as follows. The hinge and CH₃ domains were amplified from a CH₂ deleted L6 IgG1 (pNγ1.14) construct with a sense oligonucleotide (5'

CAGGGAGGGAGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide (5'GGAAAGAACCATCACAGTCTCGCAGGGG CCCAGGGCAGCGCTGGGTGCTT 3') homologous to the constant region sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pNγ1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH₃ domains.

The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH₂ and CH₃ domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH₃ PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This construct lacks the CH₂ domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH₂-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

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EXAMPLE 3

Toxicity, localization and clearance of CH₂-deleted chimeric BR96 was tested in vivo as follows.

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Three dogs received 400 mg/m² of cBR96-A, the CH₂ deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of toxicity.

Results: A significant amount of localization of the CH₂ deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m², although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent amounts of intact and CH₂ deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
		Localization	
	#271	155	
cBR96			135
	#272	114	
	#273	126	
cBR96-A			89
	#274	52	

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Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical signs of toxicity seen at doses of 10 mg/m²), even if this difference is real, it could

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran historical frozen tissues from dogs treated with native cBR96 or F(ab)2/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A,
these data indicate that the CH₂ domain is associated with the induction of acute
gastroenteropathy, and that the removal of this domain prevents the induction of
gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')2 is not toxic in the dog model and that the toxicity is mediated by the constant region. The CH₂ deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le^Y

expressing dogs. The study used chimeric versus constant region mutant of cBR96
2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid clearance. More than enough of the cBR96-A localized to have caused toxicity.

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Discussion: The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

In man the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by antiemetics, defines it as the dose-limiting toxicity.

This toxicity is mediated in man and dog by the antibody molecule alone. At higher doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')2 molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

The CH₂ domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH₂ domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

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The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m² did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

EXAMPLE 4

The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M. Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. Bio/Technology 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96. Fab. J. Biol. Chem. 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2

- Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. Immunology. 86:319-324).
- As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH₂ constant domain of human IgG₁. Six specific amino acid residues distributed throughout the CH2 domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. Immunology. 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for Clq on IgG. Nature 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six residues. We were interested in constructing a panel of mutant CH₂ domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various in vitro methods have been described where PCR is used to simultaneously introduce distally located site-specific mutations within a gene sequence (Ho, S.N., 10 H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolpf. 1996. Simultaneous introduction of multiple mutations using overlap extention PCR. BioTechniques 22:28-30). Alternatively, an in vivo procedure termed recombination PCR (RPCR) has also successfully been used for rapidly and efficiently generating 15 distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by placing homologous ends on DNA 20 using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses E. Coli's recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. In vivo recombination is mediated through the joining of nucleotide sequences designed into the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH₂ domain.

Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. J. Biol. Chem. 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by in vivo recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by 10 placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66) into vectors pD17-hGla and pD16-hCk, to form pBR96-hGla and pBR96-hCk respectively. pD17-hG1a and pD16-hCk are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH₂-15 CH₃ domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Oiagen Gel Extraction kit (Qiagen, Chatsworth, CA).

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The strategy for introducing multiple mutations within the immunoglobulin CH₂ gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues. The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and antisense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

Each L mutation was amplified in a separate PCR reaction. The reaction conditions 10 were 250 ng intact pBR96-hGla DNA template, 10 ul of 1X Pfu buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethysulfoxide (ATCC, Rockville, MD) and 2.5 units cloned Pfu DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-20 III digested pBR96-hG1a vector, transfected into Max competent E. coli DH5a according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

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The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

To evaluate the expression of Le⁷ -binding activity of the CH₂ mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6 clones derived from the quadruple recombination reaction exhibiting the predicted 15 diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hCk DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Ley binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok, G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstom, K.-E. Hellstorm, W.D. Huse and 20 S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le^γ -reactive IgG. The spectrum of Le^γ binding activities were all similar to that of native humanized BR96 IgG indicating that the homologously recombined antibodies did not acquire any gross mutations 25 that could affect antigen binding. To confirm that the desired CH₂ mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

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was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCRgenerated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing complexly mutated protein domains as well as broadening the number and location 15 of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs	PCR	HR ^a events	Colonies	Cloning
Constructed	Fragments in		Analyzed	Efficiency ^b
	reaction			
2	2	triple	24	45%
2	3	quadruple	24	33%

^aHR-homologous recombination

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^bCloning efficiency (number of clones containing 1.4kbp insert/total number of colonies

EXAMPLE 5

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This example provides two methods for introducing site specific mutations into the 5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant region, wherein mutations are introduced using appropriately constructed oligonucleotides. The vector receiving the fragment(s) is digested with a restriction enzyme to linearize the vector. PCR amplification primers are designed so that the 5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If more than one PCR fragment is amplified, then common sequences to the two fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR fragments and with the digested vector. The fragments and vector can recombine by homologous recombination using the bacteria's recombination machinery. Bacterial colonies are selected and the DNA is analyzed by size and restriction map as a preliminary determination that the vector and fragment(s) recombined correctly. Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide sequence analysis. DNA is then introduced into mammalian cells as described for the CH2 deleted antibody, and the expressed antibody analyzed for binding and functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at residue 237 were introduced by the procedure disclosed in Example 4. The heavy chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K-E. Hellstrom, I, Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two Eco47-III restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco*47-III, (2) isolating the vector by agarose gel electrophoresis, and (3) extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco*47-III digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10 μl of 10X *Pfu* buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100 μl reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector and transfected in E.coli MAX Efficiency DH5αTM according to the

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manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD). The entire transfection reaction was plated onto LB agar plated containing $100 \, \mu g/ml$ ampicillin.

- Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- The second method for introducing site specific mutations into the CH₂ domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, supra). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent E. coli CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
- 15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.
- Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridinylated DNA was prepared using the Muta-Gene Phagemid In Vitro

 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridinylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared

by a combination of thse methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3 mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

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Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

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The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

Sens(sense)CH2 E47-3-5: CAG GGA GGG AGG GTG TCT GCT GGA AGC

20 CAG GCT CAG CGC TGA CCT CAGA

D CH2 E47-3 A (antisense): GGA AAG AAC CAT CAC AGT CTC GCA GGG GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences show sites of mutation):

Antisense CH2 L235-G237/aa: GAA GAG GAA GAC TGA CGG TGC CCC CGC GAG TTC AGG TGC TGA GG

SensCH2 L235-G237/AA: CCT CAG CAC CTG AAC TCG CGG GGG CAC CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

Antis(antisense)CH2 EKK/SSS-2: CTG GGA GGG CTT TGT TGG AGA CCG

AGC ACG ACT ACG ACT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

Antis CH2 P331/A/3: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC Sense CH2 P33/A: GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC Alternative antisense oligo to introduce Ala at 331 by site-directed mutation: CH2P331A: GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

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Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

Antis CH2 EKKP/SSA-6: GAT GGT TTT CTC GAT GGC GGC TGG GAG
GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG
15 CCA GTC CTG GTG

Sense CH2 EKKP/SSA-6: CAC CAG GAC TGG CTG AAT GGC AAG TCG
TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC
GAG AAA ACC ATC

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In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant region are marked.

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SEQUENCE LISTING

5	(1) GENERAL INFORMATION
	(i) APPLICANT: Bristol-Myers Squibb Co.
10	(ii) TITLE OF THE INVENTION: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS
	(iii) NUMBER OF SEQUENCES: 13
15	(iv) CORRESPONDENCE ADDRESS:(A) ADDRESSEE: Merchant & Gould(B) STREET: 11150 Santa Monica Blvd., Suite 400(C) CITY: Los Angeles
20	(D) STATE: CA (E) COUNTRY: USA (F) ZIP: 90025
25	 (v) COMPUTER READABLE FORM: (A) MEDIUM TYPE: Diskette (B) COMPUTER: IBM Compatible (C) OPERATING SYSTEM: DOS (D) SOFTWARE: FastSEQ Version 2.0
30	(vi) CURRENT APPLICATION DATA: (A) APPLICATION NUMBER: PCT/US97/ (B) FILING DATE: 01-AUG-1997
35	(C) CLASSIFICATION: (vii) PRIOR APPLICATION DATA: (A) APPLICATION NUMBER: 60/023,033
	(B) FILING DATE: 02-AUG-1996
40	(viii) ATTORNEY/AGENT INFORMATION:(A) NAME: Adriano, Sarah B(B) REGISTRATION NUMBER: 34,470(C) REFERENCE/DOCKET NUMBER: 30436.43WOU1
45	(ix) TELECOMMUNICATION INFORMATION:(A) TELEPHONE: 310-445-1140(B) TELEFAX: 310-445-9031(C) TELEX:
50	(2) INFORMATION FOR SEQ ID NO:1:
55	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 36 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear

	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:	
5	TGGCACCGAA AGCTTTCTGG GGCAGGCCAG GCCTGA	36
,	(2) INFORMATION FOR SEQ ID NO:2:	
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15	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:	
20	TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA	57
20	(2) INFORMATION FOR SEQ ID NO:3:	
25	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 55 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear	
30	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:	
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35	(2) INFORMATION FOR SEQ ID NO:4:	
40	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 30 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:	
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50	(2) INFORMATION FOR SEQ ID NO:5:	
55	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 36 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
	(ii) MOLECULE TYPE: CDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:	

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(2) INFORMATION FOR SEQ ID NO:6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 39 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6: CATGGTCACG TGGTGTGTC CTGGATGCAG GCTACTCTA (2) INFORMATION FOR SEQ ID NO:7: 20 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 49 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 25 (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7: 30 CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA (2) INFORMATION FOR SEQ ID NO:8: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 50 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 40 (ii) MOLECULE TYPE: cDNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8: GGAAAGAACC ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT 45 (2) INFORMATION FOR SEQ ID NO:9: (1) SEQUENCE DESCRIPTION: SEQ ID NO:9: (1) SEQUENCE DESCRIPTION: SEQ ID NO:9: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 8691 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	36
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(2) INFORMATION FOR SEQ ID NO:9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 8691 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	50
(A) LENGTH: 8691 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	
(B) TYPE: nucleic acid (C) STRANDEDNESS: single	
(C) STRANDEDNESS: single	
1-1	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: cDNA 55	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:	
GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGT CCTTTTTTT TAATTTTATT TTATTTTATT TTTGAGATGG AGTTTGGCGC CGATCTCC	AA 60 CG 120

				aamama*maa	CCCNTNCTTN	አርርርእርጥስጥር	180
	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CACCALAGITA	TARCCASIAIC	240
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCGC	DAGCAMAAII	CCTTTTTCCCC	300
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGITITGCGC	360
_	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATIG	ATTATTGACT	AGIIAIIAAI	420
5	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCG	CCTGGCATTA	TGCCCAGTAC	ATGACCTTAT	660
10	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	тстататаа	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
15	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
15	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA	GGTGTCCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTCGCCAGA	1260
20	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
20	ATCCAGACAC	TGTAAAGGGT	CGATTCACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTCCADACAC	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	MCC I GCAAAI	GGCCTGGTTT	COTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
	TGGACGACGG	GGGCCCATCG	GTCTTCCCCC	TGGCACCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
25	CTAGCACCAA	CCTGGGCTGC	CTCGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCGT	1620
23	GCACAGCGGC	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCCTA	CAGTCCTCAG	1680
	GGAACTCAGG	CCTCAGCAGC	GTCGTCACCG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	GACTCTACTC	CCTCAGCAGC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
	ACATCTGCAA	GGGAGGGAGG	CTCTCTCCTC	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
20	GGCCAGCACA	ATGCAGCCCC	ACTCCACCCC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
30	CATCCCGGCT	TGCCCGCCCC	AGICCAGGGC	ACCCACACAC	TCTTCTGGCT	TTTTCCCCAG	1980
	CGGAGGCCTC	GGCACAGGCT	ACTUATORIC	AGGGAGAGGG	CTGCACACAA	AGGGGCAGGT	2040
	GCTCTGGGCA	GACCTGCCAA	AGGIGCCCCI	AACCCAGGCC	CTCCCCCTGA	CCTAAGCCCA	2100
	GCTGGGCTCA	CAAACTCTCC	GAGCCATATC	CECCAGGACC	TTCTCTCCTC	CCAGATTCCA	2160
2.5	CCCCAAAGGC	CAAACTCTCC	ACTUCCTUAG	CICGGACACC	ACANANCTCA	CACATGCCCA	2220
35	GTAACTCCCA	GTAAGCCAGC	TGCAGAGCCC	AAATCIIGIG	CARCCCCCCA	CACATOCCCA	2280
	CCGTGCCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCICCAGCI	CAAGGCGGGA	CCTCCATCTC	2340
	AGAGTAGCCT	GCATCCAGGG	ACAGGCCCCA	GUCGGGTGCT	GACACGICCA CECTECCCCC	CANARCCCAA	2400
	TTCCTCAGCA	CCTGAACTCC	TGGGGGGACC	GICAGICTIC	CICITCCCC	CAAAACCCAA	2460
40	GGACACCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GIGGIGGIGG	ACGTGAGCCA	2520
40	CGAAGACCCT	GAGGTCAAGT	TCAACTGGTA	CGTGGACGGC	GIGGAGGIGC	ATAATGCCAA	2520
	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGT	GIGGICAGCG	TCCTCACCGT	2640
	CCTGCACCAG	GACTGGCTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	2700
	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGT	GGGACCCGTG	GGGTGCGAGG	-
	GCCACATGGA	CAGAGGCCGG	CTCGGCCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	2760
45	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCCTGCCC	CCATCCCGGG	2820
	ATGAGCTGAC	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCCAGCG	2880
	ACATCGCCGT	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	2940
	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	: TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	3000
	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	3060
50	ACACGCAGA	A GAGCCTCTCC	CTGTCTCCG	GTAAATGAG1	GCGACGGCCG	GCAAGCCCCC	3120
	GCTCCCCGGC	CTCTCGCGGT	CGCACGAGG	A TGCTTGGCAC	GTACCCCCTG	TACATACTTC	3180
	CCGGGCGCCC	AGCATGGAAA	TAAAGCACC	AGCGCTGCCC	TGGGCCCCTC	CGAGACTGTG	3240
	ATGGTTCTTT	CCACGGGTCA	GGCCGAGTC	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	3300
	GGGTCCCACT	r GTCCCCACAC	TGGCCCAGG	TGTGCAGGTC	TGCCTGGGCC	CCCTAGGGTG	3360
55	GGGCTCAGCG	AGGGGCTGCC	CTCGGCAGG	G TGGGGGGATTT	CCAGCGTGC	CCCTCCCTCC	3420
	AGCAGCACCT	GCCCTGGGCT	GGGCCACGG	3 AAGCCCTAGC	AGCCCCTGGC	GACAGACACA	3480
	CAGCCCCTGC	CTCTGTAGGA	GACTGTCCT	3 TTCTGTGAG	C GCCCCTGTCC	TCCCGACCTC	3540
	CATGCCCACT	r CGGGGGCATG	CCTAGTCCA'	r grgcgtagg	ACAGGCCCTC	CCTCACCCAT	3600
	CTACCCCCA	GGCACTAACC	CCTGGCTGC	CTGCCCAGC	TCGCACCCGC	ATGGGGACAC	3660

	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCCACA	3720
	CACACACTCA	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	3780
	CACCACACAC	ACACGTGCAC	GCCTCACACA.	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	3840
	CCCAGACCAG	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGACACAGG	CCCCCACGAG	3900
5	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAGG	GTGCCCCTGC	AGCCGCCACA	CACACACAGG	4020
		CCACGTCACG				-	4080
		CAGCCTCGAC					4140
		CCTTGACCCT					4200
10		CGCATTGTCT					4260
10		GGGAGGATTG					4320
		AGGCGGAAAG					
							4380
		TAAGCGCGGC					4440
1.5		CGCCCGCTCC					4500
15		AAGGGAAAAA					4560
		CCATCCCGCC					4620
		TTTTTTTTT					4680
	AAGTAGTGAG	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTGCGATTT	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	4800
20	CCCGCTGCCA	TCATGGTTCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	4860
	ATTGGCAAGA	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	4920
	AGAATGACCA	CAACCTCTTC	AGTGGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	4980
	ACCTGGTTCT	CCATTCCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGAGAAC	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
		TGCAGGAATT					5280
		TCCCAGAATA					5340
		TTGAAGTCTA					5400
30		TAAAGCTATG					5460
50		GGAACCTTAC					5520
		TAAGGTAAAT					5580
		TGTATTTTAG					5640
		TGAGGAAAAC					5700
35		CTCTCAACAT					5760
33		TTCAGAATTG					5820
		TGCTATTTAC					5880
							5940
		TTCTGTAACC					6000
40		TCCACACAGG					
40		CTTTTTAATT					6060
		TCATAATCAG					6120
		TCCCCCTGAA					6180
		CTTATAATGG					6240
4.5		CACTGCATTC					6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	6360
		TTATTGCAGC					6420
		CATTTTTTC					6480
		TCTGTATACC					6540
		TGTGAAATTG					6600
50		AAGCCTGGGG					6660
						AATCGGCCAA	6720
		GAGGCGGTTT					6780
		TCGTTCGGCT					6840
		AATCAGGGGA					6900
55		GTAAAAAGGC					6960
-						ACTATAAAGA	7020
	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	7080
	ACCGGATACC	TGTCCGCCTI	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	7140
	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	7200
	TOTHOGIATO		31	3000000			

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CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA
     AGACACGACT TATCGCCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT
                                                                         7320
     GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA
     GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT
     TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT
     ACGCGCAGAA AAAAAGGATC TCAAGAAGAT CCTTTGATCT TTTCTACGGG GTCTGACGCT
     CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC
     ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA
     ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA
     TTTCGTTCAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC
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     TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT
     TTATCAGCAA TAAACCAGCC AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA
     TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT
     AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTCACG CTCGTCGTTT
     GGTATGGCTT CATTCAGCTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG
                                                                         8100
15
     TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC
     GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC
                                                                         8220
     GTAAGATGCT TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG
     CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGCC ACATAGCAGA
     ACTITAAAAG IGCICATCAT IGGAAAACGI ICTICGGGGC GAAAACTCIC AAGGAICTIA
20
     CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT
                                                                         8460
     TTTACTTTCA CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAAATGC CGCAAAAAAG
                                                                         8520
     GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA
     AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT
                                                                         8640
     AAACAAATAG GGGTTCCGCG CACATTTCCC CGAAAAGTGC CACCTGACGT C
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(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8327 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TRATTTTATT	TTATTTTATT	TTTGAGATGG	AGTTTGGCGC	CGATCTCCCG	120
	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT				TTGACGTCAA		540
	ATTTACGGTA	AACTGCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCG			ATGACCTTAT	660
50	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	·780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
55	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA	GGTGTCCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTCGCCAGA	1260

						ATAACCGACT	1320
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	ACCTGCAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
5	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCGT	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCCTA	CAGTCCTCAG	1680
		CCTCAGCAGC					1740
		CGTGAATCAC				•	1800
10		GGGAGGGAGG					1860
		ATGCAGCCCC					1920
		TGCCCGCCCC					1980
		GGCACAGGCT					2040
		GACCTGCCAA					
15							2100
13		CAAACTCTCC					2160
		ATCTTCTCTC					2220
		GTAAGCCAGC					2280
		GCATCCAGGG					2340
20		CTCGGCCCAC					2400
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		GTCAGCCTGA					2520
	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	CCGTGCTGGA	2580
	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	GGTGGCAGCA	2640
_	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGCAGAA	2700
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	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCCTG	TACATACTTC	CCGGGCGCCC	2820
	AGCATGGAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCCTG	CGAGACTGTG	ATGGTTCTTT	2880
	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	2940
	GTCCCCACAC	TGGCCCAGGC	TGTGCAGGTG	TGCCTGGGCC	CCCTAGGGTG	GGGCTCAGCC	3000
30	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT	3060
	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCCTGGG	GACAGACACA	CAGCCCCTGC	3120
	CTCTGTAGGA	GACTGTCCTG	TTCTGTGAGC	GCCCCTGTCC	TCCCGACCTC	CATGCCCACT	3180
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		CCTGGCTGCC					3300
35		ACTCTCGGGC					3360
	•	GTTCAACAAA					3420
		GCCTCACACA					3480
		TCGCACACGT					3540
		CCCACGAGCC					3600
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~ 0						CTAACTCCGC	4200
50						TGACTAATTT	
						AAGTAGTGAG	4320
						GCTGCGATTT	4380
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55	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	AGAATGACCA	4560
	CAACCTCTTC	AGTGGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	ACCTGGTTCT	4620
	CCATTCCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC	4680
	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	GCCTTAAGAC	4740
						GGAGGCAGTT	4800

	concentration and a	GGAAGCCATG	DATCABCCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA	4860
	CIGITIACCA	TGAAAGTGAC	ACCITITUTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	4920
	TGCAGGAATT	CCCAGGCGTC	CTCTCTCTCTCC	TCCACCACCA	DADAGGCATC	AAGTATAAGT	4980
	TCCCAGAATA	CCCAGGCGTC	CICICIGAGG	A S C S TO COTTON	CARCTTCTCT	CCTCCCCTCC	5040
_	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGAIGCIII	COMBILCICI	TOTTTOTON	5100
5	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTITICCIG	CCTACACACA	TTTTDAGCTC	5160
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	TAAGGTAAAT	TTTTAAAATA	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	AMACCOMMUNA	5280
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	TGAGGAAAAC	CTGTTTTGCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA	5340
10	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTITCC	5400
	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAACTC	TTGCTTGCTT	5460
	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	TGGAAAAATA	5520
	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC	5580
	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	GTACCTTTAG	5640
15	CTTTTTAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	TGACTAGAGA	5700
	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAAC	CTCCCACACC	5760
	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	5820
	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTTTT	5880
	CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
20	CCTCGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	CCCAACTTGT	6000
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	CATTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG	6120
	TOTOTATACO	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG	6180
	TCTCANATTC	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGTA	6240
25	1GIGAAAIIG	TGCCTAATGA	CTCACCTAAC	TCACATTAAT	TGCGTTGCGC	TCACTGCCCG	6300
23	AAGCCTGGGG	GGGAAACCTG	TCCTCCCACC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	6360
	CTTTCCAGTC	GCGTATTGGG	CCCTCTTCC	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	6420
	CAGGCGGTTT	GCGGCGAGCG	CTATCACCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	6480
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30	GTAAAAAGGC	CTCAAGTCAG	ACCTCCCGAA	ALAGGETEEG	ACTATAAAGA	TACCAGGCGT	6660
	AAAATCGACG	AAGCTCCCTC	AGGIGGCGAA	CTCTTCCCAC	CCTGCCGCTT	ACCGGATACC	6720
	TTCCCCCTGG	AAGCTCCCTC	GIGCGCICIC	CIGIICCGAC	ATCCTCACCC	TGTAGGTATC	6780
	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG	TO CONTINUE TO THE TOTAL TOTAL TO THE TOTAL	AIGCICACGC	CCCCTTCAGC	6840
25	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	CARCCACCC	ACACACGACT	6900
35	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	ACCCACCTAT	CTACCCCCC	6960
	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGIAI	CTATTTCCTA	7020
	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	TCATCCCCCA	7080
	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	1GATCCGGCA	7140
	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	7200
40	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGIGGAACG	7260
	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCIAGAICC	
	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTIGGICIG	7320
	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTCAT	7380
	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG	7440
45	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	7500
	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGC	GAAGTGGTCC	TGCAACTTTA	TCCGCCTCCA	7560
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	CATTCAGCTO	CGGTTCCCAA	CGATCAAGG	C GAGTTACATG	ATCCCCCATC	TTGTGCAAAA	7740
50	AAGCGGTTAC	CTCCTTCGGT	CCTCCGATC	TTGTCAGAAG	TAAGTTGGCC	GCAGTGTTAT	7800
	CACTCATGGT	TATGGCAGCA	CTGCATAAT?	r ctcttactgt	CATGCCATCC	: GTAAGATGCT	7860
	TTTCTGTGAG	TGGTGAGTAC	TCAACCAAG	r cattctgaga	ATAGTGTATG	CGGCGACCGA	7920
	GTTGCTCTTC	CCCGGCGTCA	ATACGGGAT	A ATACCGCGCC	: ACATAGCAGA	A ACTTTAAAAG	7980
	TGCTCATCAT	r TGGAAAACGT	TCTTCGGGG	C GAAAACTCTC	: AAGGATCTTA	CCGCTGTTGA	8040
55	GATCCAGTTC	GATGTAACCC	ACTCGTGCA	CCAACTGATC	: TTCAGCATC	TTTACTTTCA	8100
	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGA	A GGCAAAATGC	CGCAAAAAA	GGAATAAGGG	8160
	CGACACGGA	ATGTTGAATA	CTCATACTC	T TCCTTTTTCA	ATATTATTG	AGCATTTATC	8220
	AGGGTTATT	TOTOATGAGO	GGATACATA	TTGAATGTAT	TTAGAAAAA	r AAACAAATAG	8280
	GGGTTATIC	CACATTTCCC	CGAAAAGTG	C CACCTGACGT	CCBRAAG		8327
	999116696	, charrie	. 50,238,010				

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8897 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGCAC CATGAAGTTG CCTGTTAGGC 15 TGTTGGTGCT GATGTTCTGG ATTCCTGCTT CCAGCAGTGA TGTTTTGATG ACCCAAATTC 120 CAGTCTCCCT GCCTGTCAGT CTTGGAGATC AAGCGTCCAT CTCTTGCAGA TCTAGTCAGA 180 TCATTGTACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT 240 CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCCA GACAGGTTCA GCGGCAGTGG ATCAGGGACA GATTTCACAC TCAAGATCAG CAGAGTGGAG GCTGAGGATC TGGGAGTTTA TTACTGCTTT CAAGGTTCAC ATGTTCCATT CACGTTCGGC TCGGGGACAA 20 420 AGTTGGAAAT AAAACGTAAG TCTCGAGTCT CTAGATAACC GGTCAATCGA TTGGAATTCT AAACTCTGAG GGGGTCGGAT GACGTGGCCA TTCTTTGCCT AAAGCATTGA GTTTACTGCA 540 AGGTCAGAAA AGCATGCAAA GCCCTCAGAA TGGCTGCAAA GAGCTCCAAC AAAACAATTT AGAACTTTAT TAAGGAATAG GGGGAAGCTA GGAAGAAACT CAAAACATCA AGATTTTAAA TACGCTTCTT GGTCTCCTTG CTATAATTAT CTGGGATAAG CATGCTGTTT TCTGTCTGTC 25 720 CCTAACATGC CCTTATCCGC AAACAACACA CCCAAGGGCA GAACTTTGTT ACTTAAACAC 780 CATCCTGTTT GCTTCTTCC TCAGGAACTG TGGCTGCACC ATCTGTCTTC ATCTTCCCGC CATCTGATGA GCAGTTGAAA TCTGGAACTG CCTCTGTTGT GTGCCTGCTG AATAACTTCT 900 ATCCCAGAGA GGCCAAAGTA CAGTGGAAGG TGGATAACGC CCTCCAATCG GGTAACTCCC 960 AGGAGAGTGT CACAGAGCAG GAGAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCCTGA 30 CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACGC CTGCGAAGTC ACCCATCAGG GCCTGAGCTC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAGAGG GAGAAGTGCC 1140 CCCACCTGCT CCTCAGTTCC AGCCTGACCC CCTCCCATCC TTTGGCCTCT GACCCTTTTT CCACAGGGGA CCTACCCCTA TTGCGGTCCT CCAGCTCATC TTTCACCTCA CCCCCCTCCT CCTCCTTGGC TTTAATTATG CTAATGTTGG AGGAGAATGA ATAAATAAAG TGAATCTTTG 35 1320 CACCTGTGGT TTCTCTCTTT CCTCATTTAA TAATTATTAT CTGTTGTTTT ACCAACTACT CAATTTCTCT TATAAGGGAC TAAATATGTA GTCATCCTAA GGCACGTAAC CATTTATAAA AATCATCCTT CATTCTATTT TACCCTATCA TCCTCTGCAA GACAGTCCTC CCTCAAACCC 1500 ACAAGCCTTC TGTCCTCACA GTCCCCTGGG CCATGGTAGG AGAGACTTGC TTCCTTGTTT 1560 40 TCCCCTCCTC AGCAAGCCCT CATAGTCCTT TTTAAGGGTG ACAGGTCTTA CAGTCATATA TCCTTGATT CAATTCCCTG AGAATCAACC AAAGCAAATT TTTCAAAAGA AGAAACCTGC TATAAAGAGA ATCATTCATT GCAACATGAT ATAAAATAAC AACACAATAA AAGCAATTAA ATAAACAAAC AATAGGGAAA TGTTTAAGTT CATCATGGTA CTTAGACTTA ATGGAATGTC ATGCCTTATT TACATTTTTA AACAGGTACT GAGGGACTCC TGTCTGCCAA GGGCCGTATT GAGTACTTTC CACAACCTAA TTTAATCCAC ACTATACTGT GAGATTAAAA ACATTCATTA 1920 45 AAATGTTGCA AAGGTTCTAT AAAGCTGAGA GACAAATATA TTCTATAACT CAGCAATCCC 1980 ACTTCTAGAT GACTGAGTGT CCCCACCCAC CAAAAAACTA TGCAAGAATG TTCAAAGCAG CTTTATTTAC AAAAGCCAAA AATTGGAAAT AGCCCGATTG TCCAACAATA GAATGAGTTA 2100 TTAAACTGTG GTATGTTTAT ACATTAGAAT ACCCAATGAG GAGAATTAAC AAGCTACAAC 2160 TATACCTACT CACACAGATG AATCTCATAA AAATAATGTT ACATAAGAGA AACTCAATGC 2220 50 AAAAGATATG TTCTGTATGT TTTCATCCAT ATAAAGTTCA AAACCAGGTA AAAATAAAGT 2280 TAGAAATTTG GATGGAAATT ACTCTTAGCT GGGGGTGGGC GAGTTAGTGC CTGGGAGAAG 2340 ACAAGAAGGG GCTTCTGGGG TCTTGGTAAT GTTCTGTTCC TCGTGTGGGG TTGTGCAGTT 2400 ATGATCTGTG CACTGTTCTG TATACACATT ATGCTTCAAA ATAACTTCAC ATAAAGAACA 2460 TCTTATACCC AGTTAATAGA TAGAAGAGGA ATAAGTAATA GGTCAAGACC AACGCAGCTG 2520 55 GTAAGTGGGG GCCTGGGATC AAATAGCTAC CTGCCTAATC CTGCCCWCTT GAGCCCTGAA CATCTGTGCC CTGTTTGGCT AGCTAGGAGC ACACATACAT AGAAATTAAA TGAAACAGAC 2700 CTTCAGCAAG GGGACAGAGG ACAGAATTAA CCTTGCCCAG ACACTGGAAA CCCATGTATG

						######################################	2820
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	ATGGGGCACT	CTGGCCCTGC	CCCTCTCAGC	TACTCATCCA	TCCAACACAC	CTTTCTAAGT	2880
	ACCTCTCTCT	GCCTACACTC	TGAAGGGGTT	CAGGAGTAAC	TAACACAGCA	TCCCTTCCCT	2940
	CAAATGACTG	ACAATCCCTT	TGTCCTGCTT	TGTTTTTCTT	TCCAGTCAGT	ACTGGGAAAG	3000
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	TTGACCCTGG	AAGGTGCCAC	TCCCACTGTC	CTTTCCTAAT	AAAATGAGGA	AATTGCATCG	3420
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	CACCATTEGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGCTTCTGAG	3540
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15	*CCCCCCCCCCC	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA	CACTTGCCAG	CGCCCTAGCG	3660
13	AGCGCGGCGG	TCGCTTTCTT	CCCTTCCTTT	CTCGCCACGT	TCGCCGGGCC	TCTCAAAAAA	3720
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	ATCCCGCCCC	CAGAGGCCGA	CAGTICCOCC	CCCTCTCACC	TATTCCAGAA	GTAGTGAGGA	3900
•	TTTATTTATG	GAGGCCTAGG	GGCCGCCTCG	B B COTTO C B C	ACCTCACGGC	TGCGATTTCG	3960
20	GGCTTTTTTG	GAGGCCTAGG	CTTTTGCAAA	AAGCTIGGAC	AGCICAGGGC	COCTOCONTO	4020
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                                                                           8897
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45
               (2) INFORMATION FOR SEQ ID NO:12:
            (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 8321 base pairs
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              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: cDNA
55
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
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60

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA

TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG TGGTTAAGCT TGGTCTTCCT

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5	CACACACAAT	GCAAAGAACA	CCCTGTACCT	GCAAATGAAC	AGCCTGAGGG	ACGAGGACAC	420
5	CAGAGACAAI	TACTGTGCAA	CACCCCTCCC	GGACGGGGCC	TEGTTTGCTT	ACTGGGGCCA	480
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10	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TCACCGIGCC	720
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	CAAGGTGGAC	AAGAAAGTTG	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	840
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20
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             (A) LENGTH: 8897 base pairs
25
              (B) TYPE: nucleic acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: cDNA
30
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
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What is claimed is:

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1. A method for inhibiting immunoglobulin-induced toxicity resulting from

immunoglobulin immunotherapy in a subject comprising administering an

immunoglobulin molecule to the subject, the immunoglobulin molecule

having a variable region and a constant region, the immunoglobulin molecule

being modified prior to administration by structurally altering multiple

toxicity associated domains in the constant region so that immunoglobulin
induced toxicity is inhibited.

2. A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering a structurally altered antibody to the subject, the structurally altered antibody comprising a variable region and a constant region, multiple toxicity associated domains in the constant region being modified so as to render the constant region unable to mediate an ADCC response or activate complement thereby inhibiting immunoglobulin-induced toxicity resulting from immunotherapy.

3. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein having multiple structurally altered toxicity

associated domains in the constant region.

4. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein comprising a modified constant region, the

modification being a structural alteration in multiple toxicity associated regions within the CH₂ domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:

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- selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
- 10 (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
- 15 (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
 - 6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- 25 (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
 - (b) structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the lg protein so selected;

(c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH₂ domain thereby preventing immunoglobulin-induced toxicity in the subject.

7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH₂ domain.

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- 8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
- 9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
 - 10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
 - 11. The method of claim 2, wherein the antibody recognizes and binds Le^y.
- 20 12. The method of claim 2, wherein the antibody recognizes and binds to Lex.
 - 13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
 - 14. The method of claim 2, wherein the antibody is a chimeric antibody

 ChiBR96 produced by the hybridoma having the identifying characteristics

 of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le^y.

- The method of claim 1 or 5, wherein the immunoglobulin recognizes and
 binds to Le^x.
 - 17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.

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- 18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
- 15 19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le^y.
 - 20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le^x.

- 21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25 22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
 - 23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

- A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.
- 10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.
- The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 27. The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.
 - 29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.

31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.

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- 32. A method for treating a subject suffering from a cancer, the cancer being characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound thereby curing the subject.
 - 33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH₂ domain.
- 34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
 - 35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:

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- (a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and
- (b) administering the structurally altered BR96 polypeptide of step (a) to
 the subject under conditions so that the peptide recognizes and binds
 cancer associated Le^y antigens, thereby alleviating symptoms
 associated with the cancer, the structural alteration of the toxicity
 associated domains thereby preventing BR96 toxicity in the subject.
- 25 37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.

- 39. A BR96 antibody having humanized variable and constant regions, wherein the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
- 40. The BR96 antibody of claim 39 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 12.
 - 41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.

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42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

- 43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
- 25 44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

- 45. A BR96 antibody designated hBR96-2F having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
- 46. A BR96 antibody designated hBR96-2G having a structurally altered

 constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
- 15 47. A BR96 antibody designated hBR96-2H having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
 - 48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
 - 49. A cDNA of claim 48.

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50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.

52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein so produced.

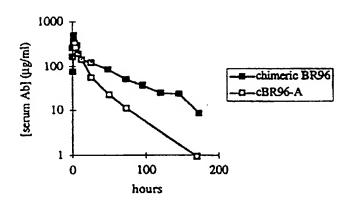


Figure 1. Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

Figure One

Figure 2

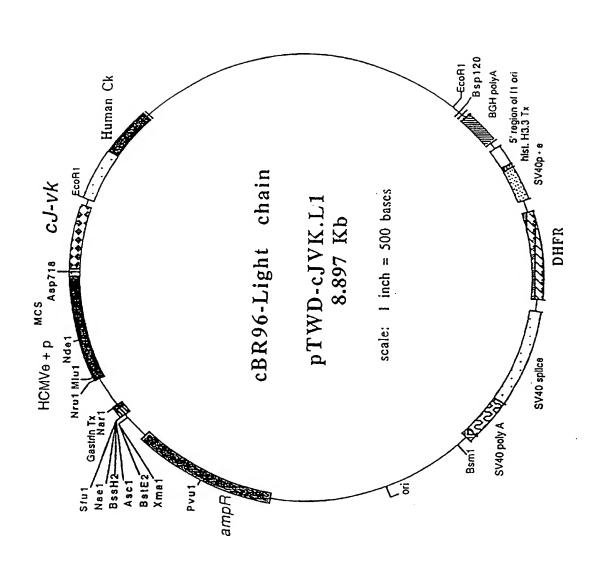
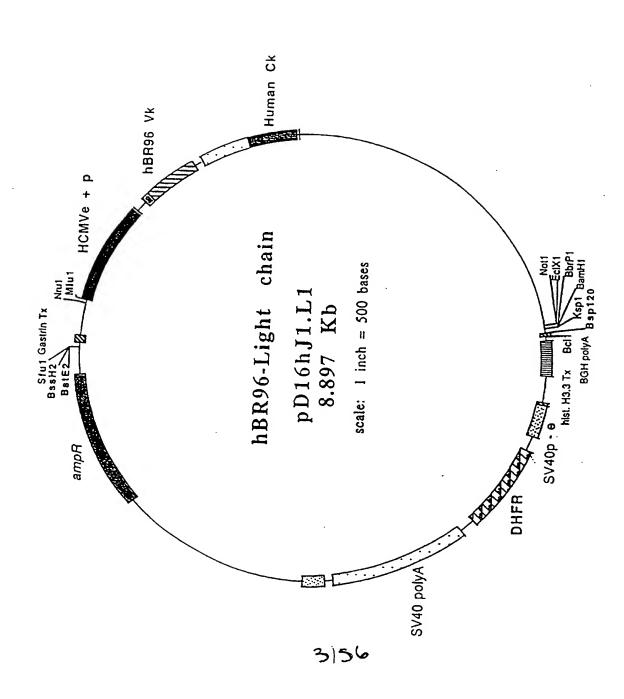


Figure 3



PCT/US97/13562

Figure 4

WO 98/05787

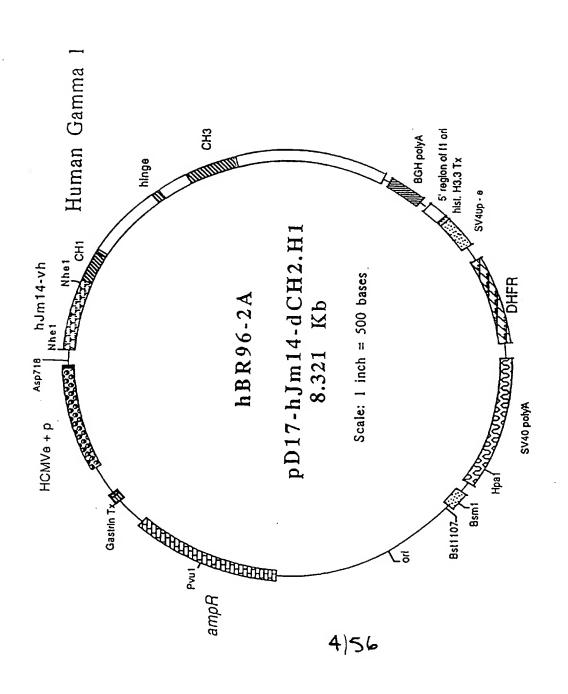


Figure 5

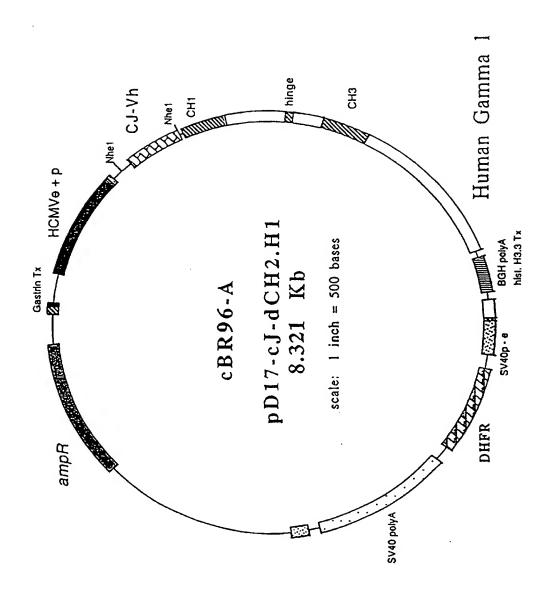


Figure 6

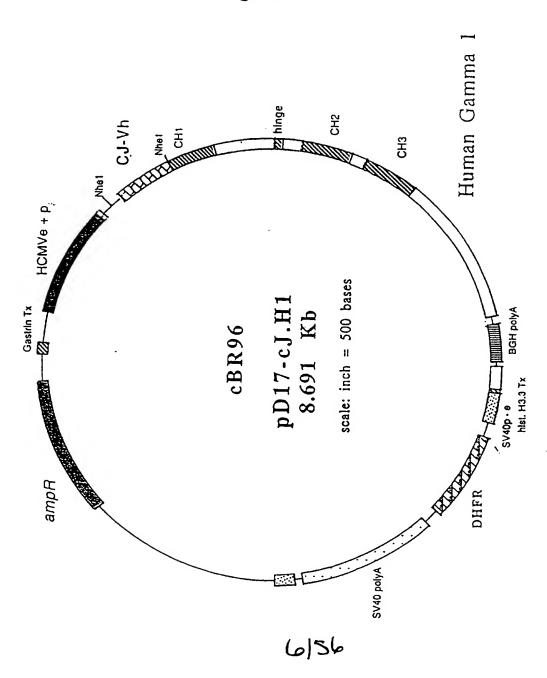


Figure 7

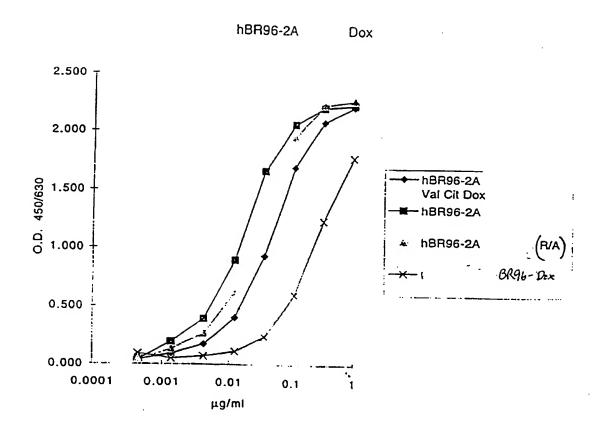
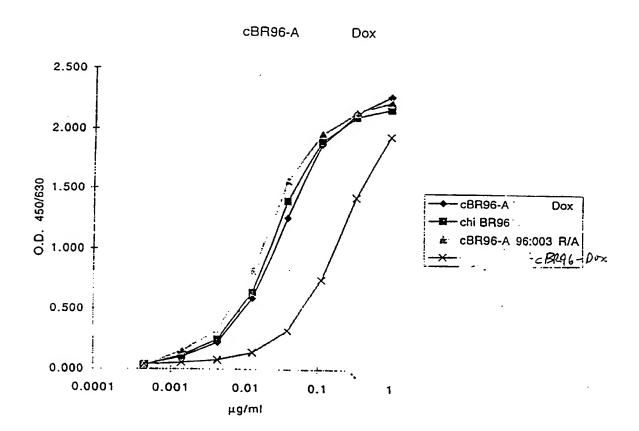
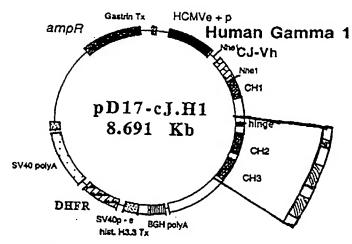


Figure 8



A- Hinge + CL_ + 'H3 domains were removed from \R96 IgG1 construct by E.co -III restriction digestion.



3. 2 - Hinge + CH3 domains amplified by PCR from L6 IgG1 construct lacking the CH2 domain.

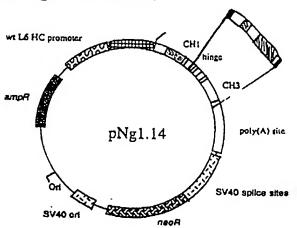


Figure 9

#3 - Hinge +CH3 PCR fragment cloned by homologous recombination into E.co47-III site of BR96 IgG1 molecule.

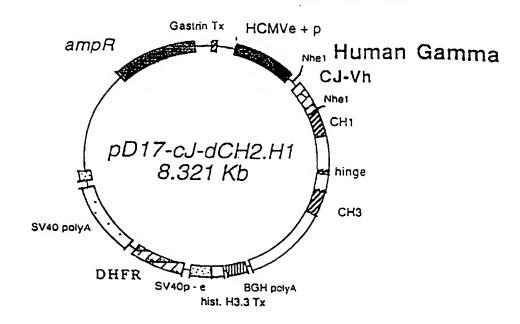
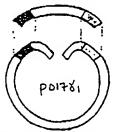


Figure 9
(CONTINUED)

- 1- Introduction of mutations by site-directed mutagenesis on double-stranded plasmid DNA.
- A- Mutations introduced into synthetic oligonucleotides used for the PCR amplification of CH2 domain.

B- Plasmid DNA linearized inside CH2 domain and cotransformed with PCR fragment into competent DH5 α .



C- Cloning mediated by homologous recombination yields transformants harbouring recombinant plasmids.

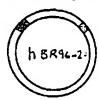
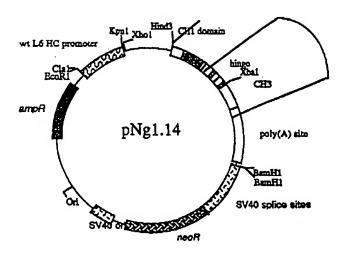


Figure 10

Figure 11



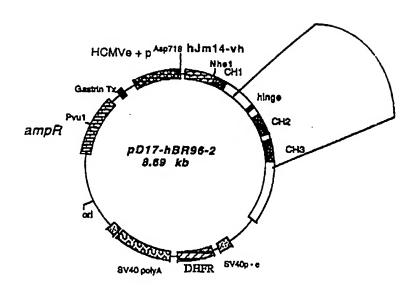


Figure 12

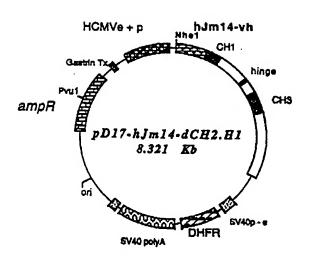


Figure 13

pD17-cJ-dCH2.H1

90	180	270	360	450	540	630	720	810	900
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AATAAAATAA	TCGGTCATAG	TTAACGTACT	TCAATAATTA	CCGACTGGCG	ACCCACCTGA	TYTACCGGGC	TACCACTACG	CCCTCAAACA	GCCACCTCC
80	170	250 260	350	440	530	620	720	800	890
TAATTTTATT	CGCATAGTTA	CAAGGCAAGG CTIGACCGAC	ATTATTGACT	TGGCCCGCCT GGCTGACCGC	TTGACGTCAA	CAATGACGGT	TATTAGTCAT CGCTATTACC ATGGTGATGC	TGACGTCAAT	TAGGCGTGTA
ATTAAAATAA	GCGTATCAAT	GTTCCGTTCC GAACTGGCTG	TAATAACTGA	ACCGGGCGGA CCGACTGGCG	AACTGCAGTT	GTTACTGCCA	ATAATCAGTA GCGATAATGG TACCACTACG	ACTGCAGTTA	ATCCGCACAT
70 CCTTTTTTTT GGAAAAAAA	160 GCTCTGATGC CGAGACTACG	250 CAAGGCAAGG GTTCCGTTCC	340 GTTGACATTG CAACTGTAAC	TTACGGTAAA AATGCCATTT	520 GGACTTTCCA CCTGAAAGGT	610 CTATTGACGT GATAACTGCA	700 TATTAGTCAT ATAATCAGTA	790 TCCACCCCAT AGGTGGGGTA	880 AAATGGGCGG TTTACCCGCC
60	150	240	330	420	510	600	690	770 780 790 CTCACCCCAT GAGGGCCCCAT GAGGGTCAG AGGTGGGGTA	870
GCCAGAGTAA	AGTACAATCT	TAAGCTACAA	AGATATACGC	GTTACATAAC	ACGCCAATAG	AGTACGCCCC	TACATCTACG		CCATTGACGC
CGGTCTCATT	TCATGTTAGA	ATTCGATGTT	TCTATATGCG	CAATGTATTG	TGCGGTTATC	TCATGCGGGG	ATGTAGATGC		GGTAACTGCG
50	140	230	320	410	500	590	680	770	860
GCTTCGAATA	GTCGACTCTC	GAGCAAAATT	TGTACGGGCC	GGAGTTCCGC	TCCCATAGTA	TCATATGCCA	TACTTGGCAG	CTCACGGGGA	CAACTCCGCC
CGAAGCTTAT	CAGCTGAGAG	CTCGTTTTAA	ACATGCCCGG	CCTCAAGGCG	AGGGTATCAT	AGTATACGGT	ATGAACCGTC	GAGTGCCCCT	GTTGAGGCGG
10 20 30 40 50 60 GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCG GCTTCGAATA GCCAGAGTAA CCTTTTTTT TAATTTTATT TTATTTTATT	100 110 110 120 130 140 150 150 140 150 150 170 180 150 170 180 170 180 170 180 170 180 180 180 180 180 180 180 180 180 18	190 200 220 220 230 240 250 260 270 270 240 250 260 270 270 270 270 270 270 270 270 270 27	280 340 350 350 350 350 320 320 330 330 340 350 350 340 350 340 340 350 340 340 350 340 350 340 350 340 350 340 350 340 350 350 340 350 350 350 350 350 350 350 350 350 35	390 430 420 430 AGTAATCATA GCCCATATAT GCAGTTCCGC GTTACATAAC TTACGGTAAA TCATTAGTTA ATGCCCCAGT AATCAATAT CGGGTATATA CCTCAAGGCG CAATGTATTG AATGCCATTT	470 480 510 510 520 530 540 CGGCCCATTG ACGCCCATTG GGACTTTCCA TTGACGTCAA TGGGTGGACT GGGGGGGATATT ACTGCATACA AGGSTATCAT TGCGGTTATC CCTGAAAGT ACTGCATACA AGGSTATCAT TGCGGTTATC CCTGAAAGGT AACTGCAGTATA AGGSTATCAT TGCGGTTATC CCTGAAAGGT AACTGCAGTATA AGGSTATCAT TGCGGTTATC CCTGAAAGGT AACTGCAGTATA AGGSTATCAT TGCGGTTATC CCTGAAAGGT AACTGCAGTATA AGGCTACTGAA	S50 550 500 500 500 570 580 590 600 600 610 620 630 810 ATTRACEGIA ARCTIGICAC TYCICACTAT ATTRACEGIA ARTIGICAC TYCICACTATE TAGITICACAT AGTACACAT AGTATICACAT TAGITICACAT TYCICACT AGTATICACAT AGTATICACAT TAGITICACAT AGTATICACAT AGTATICACGAT AGTATICACGAT AGTATICACAT AGTATICACGAT AGTATATAT AGTATICACGAT AGTATICACGAT AGTATICACGAT AGTATICACGAT AGTATICACAT AGTATI	640 650 660 710 720 CCTGGCATTA GGGACTTTC TACTTGGCAG TACATCTACG TATTAGTCAT GGGTATTACC ATGGTGATGC GGACCGTAT ACGGGTCATG TACTGATAC ATGAGAGTA CCCTGAAAGG ATGAACGGTC ATGTAGATGC ATGCAGAATA ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACGGTC ATGTAGAATGC ATGTAGAATA ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACGGTC ATGTAGAATGC ATGTAGAATA ACGGGTCATA ACGGGTCATG TACTGGAATA ACGGAATA ACGGGTCATA ACGGGTCATG ATGAAAGG ATGAACGGTC ATGTAGAATGC ATGTAGAATGC ATGAAACGGTC ATGTAGAATGC ATGAAACGGTC ATGTAGAATGC ATGTAGAATGC ATGAAACGGTC ATGTAGAATGC ATGTAGAATGAATGC ATGTAGAATGC ATGTAGAATGC ATGTAGAATGC ATGTAGAATGC ATGTAGAATGAATGC ATGTAGAATGC ATGTAGAATGC ATGTAGAATGC ATGTAGAATGAATGAATGAATGAATGAATGAAATGA	730 740 800 800 800 6GTTTGA CTCACGGGGA ITTCCAAGTC TCCACCCCAT TGACGTCAAT GGGGTTTGA CTCAAGGGA ITTCCAAGTC TCACCCCAT TGACGTCAAT GGGGTTTGT CCAAAACCGT CATGTAGTTA CCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA	820 830 830 840 850 860 860 870 900 900 870 870 880 870 880 880 890 900 900 TAGGCGTGTA CGGTGGAGG AAATGGCGG TAGGCGTGTA CGGTGGAGG AAACGGCGG TAGGCGTGTA CGGTGGAGG AAACGGCG TATAGTTGCC CATCGCACAT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCGGCACAT GCCACCCTCC
30	120	210	300	390	480	570	660	750	840
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TCCACTGGAC	GCTAGAGGGC	CTCCAGCGAC	GCAAAACGCG	AATCAAGTAT	TGCAGTTATT	AACCGTCATG	TACTGGAATA	CCCGCACCTA	CTCAAAGGTT
20	110	200	290	380	460 470 CCAACGACCC CCGCCCATTG GGTTGCTGGG GGCGGGTAAC	560	650	740	830
GAGATCTGCT	AGTTTGGCGC	TTGTGTGTTG	TAGGGTTAGG	TACGGGGTCA		AACTGCCCAC	TGCCCAGTAC	GTACATCAAT	AATCAACGG
CTCTAGACGA	TCAAACCGCG	AACACACAAC	ATCCCAATCC	ATGCCCCAGT		TTGACGGGTG	ACGGGTCATG	CATGTAGTTA	TTTAGTTGCC
10	100	190	280	370	460	550	640	730	820
GACGGATCGG	TTTGAGATGG	TGCTCCCTGC	AGAATCTGCT	AGTAATCAAT	CCAACGACCC	ATTTACGGTA	CCTGGCATTA	GGTTTTGGCA	TYTGGCACCA
CTGCCTAGCC	AACTCTACC	ACGAGGGACG	TCTTAGACGA	TCATTAGTTA	GGTTGCTGGG	TAAATGCCAT	GGACCGTAAT	CCAAAACCGT	AAACCGTGGT

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980 TCACTATAGG AGTGATATCC 1070 TCTTGCGGCC AGAACGCCGG	1140 1150 1150 1160 1170 GGTGTCCAGT GTGAAGTGAA TCTCGTCGAG TCTCGGGGAG CCACAGGTCA CACTTCACTT AGACCACCTC AGACCCCCTC	1250 CATGTATTGG GTACATAACC	1340 TCTAAAGGGT ACATTTCCCA	1430 GTATTACTGT CATAATGACA	1520 GGGCCCATCG CCCGGGTAGC	1610 CGAACCGGTG GCTTGGCCAC	1700 CCTCAGCAGC GGAGTCGTCG	1790 GGACAAGAAA CCTGTTCTTT
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960 CTTATCGAAA GAATAGCTTT 1050 ACCGGTCAAT TGGCCAGTTA	1140 GGTGTCCAGT CCACAGGTCA	1230 TTCACTTTCA AAGTGAAAGT	1320 ATAACCGACT TATTGGCTGA	1410 AAGTCTGAGG TTCAGACTCC	1500 GTCTCTGTAG CAGAGACATC	1590 CTGGTCAAGG GACCAGTTCC	1680 CAGTCCTCAG GTCAGGAGTC	1770 AAGCCCAGCA TTCGGGTCGT
950 TGCTTACTGG ACGAATGACC 1040 TCTCTAGATA AGAGATCTAT	1130 TGTTTTAAAA ACAAAATTTT	1220 AACCTCTGGA TTGGAGACCT	1310 AGGTGGTGAT TCCACCACTA	1400 GAGCCGTCTG CTCGGCAGAC	1490 TCTGGTCACG AGACCAGTGC	1580 CCTGGGCTGC GGACCCGACG	1670 GGCTGTCCTA CCGACAGGAT	1760 CGTGAATCAC GCACTTAGTG
910 920 930 940 950 960 960 970 950 960 970 980 990 990 990 990 990 990 990 990 99	1090 1100 1110 1120 1130 1130 1140 1150 1160 1170 CACCATGGGG TYGTGGGTGCT TGTTTTAAA GGTGTCCAGT GTGAAGTGAA	1180 1190 1250 1210 1220 1220 1230 1250 1250 1250 1250 1250 1250 1250 125	1310 1320 1340 1350 1350 1350 1310 1320 1330 1330 1340 1350 1350 1340 1350 1350 1350 1350 1350 1350 1350 135	1360 1370 1370 1330 1400 1410 1420 1430 1430 1440 TCTCCAGAGA CAATGCCAAG AACACCCTGT ACCTGCAAAT GAGCCGTCTG AAGTCTGAGG ACACAGCCAT GTATAATGT GCAAGAGGCC AGAGGTCTT GTTACGGTTC TYGTGGGACA TGGACGTTTA CTCGGCAGAG TYCAGACTAC TGTGTCGGTA CATAATGACA CGTTCTCCGG	1450 1460 1570 1570 1530 1570 1530 1530 1530 1530 1530 1530 1530 153	1540 1550 1610 1650 1650 1670 1680 1580 1690 1600 1610 1620 1600 1610 1620 1600 1610 1620 1600 160	1610 1640 1650 1660 1670 1670 1680 1700 1700 1710 GGAACTCAGG CACCCTGAGCAGC GTGGTCAGCG GTGGTCAGCG CCTCAGCAGC GTGGTCAGCG CCTTGAGTCC CCTCAGCAGC GTGGTCAGCGC CCTTGAGTCC CCGGGACTG TGTGGAAGGG CCQACAGGAT GTCAGGAGTC CTGAGATGA GAAGTCGTCG CACCAGTGGC	1720 1730 1740 1750 1760 1800 1800 1800 1800 1800 1800 1800 18
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920 CAGAGCTCTC GTCTCGAGAG 1010 ATTTAAATTG	1100 TTGTGGTTAA AACACCAATT	1190 GCCTGGAGGG CGGACCTCCC	1280 GAGGCTGGAG CTCCGACCTC	1370 CAATGCCAAG GTTACGGTTC	1460 GGCCTGGTTT CCGGACCAAA	1550 CTCCAAGAGC GAGGTTCTCG	1640 CGCCCTGACC GCGGGACTGG	1730 CAGCTTGGGC GTCGAACCCG
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1890 AGTCCAGGGC TCAGGTCCCG	1980 TTTTCCCCAG AAAAGGGGTC	2070 GAGCCATATC CTCGGTATAG	2160 CCAGATTCCA GGTCTAAGGT	2250 CCAGGCCTCG GGTCCGGAGC	2340 GCCACATGGA CGGTGTACCT	2430 CACAGGTGTA GTGTCCACAT	2520 ACATCGCCGT TGTAGCGGCA	2610 TCTACAGCAA AGATGTCGTT	2700 ACACGCAGAA TGTGCGTCTT
1880 ATGCAGCCCC TACGTCGGGG	1970 TCTTCTGGCT AGAAGACCGA	2040 2050 2060 AGGGGCAGOT GCTGGGCTCA GACCTGCCAA TCCCCGTCCA CGACCGGGTT	2140 2150 CTCGGACACC TTCTCTCCTC GAGCCTGTGG AAGAGAGGAG	2240 GTAAGCCAGC CATTCGGTCG	2330 CATGTCCGGA GTACAGGCCT	2420 CCCCGAGAAC GGGGCTCTTG	2490 2500 2510 CCTGCCTGGT CAAAGGCTTC TATCCCAGCG GGACGGACCA GTTTCCGAAG ATAGGGTCGC	2580 2590 2600 CCGTCCTGGA CTCCGACGCC TCCTTCTCC GCCACGACCT GAGGCTGCCG AGGAAGAAGG	2690 CACAACCACT GTGTTGGTGA
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1850 TCAGCGCTCC AGTCGCGAGG	1940 TGCCCGCCCC ACGGGGGGGG	2030 CTGCACACAA GACGTGTGTT	2120 CAAACTCTCC GTTTGAGAGG	2210 ACAAAACTCA TGTTTTGAGT	2300 GCATCCAGGG CGTAGGTCCC	2390 GCTGTACCAA CGACATGGTT	2480 GTCAGCCTGA CAGTCGGACT	2570 ACCACGCCTC TGGTGCGGAG	2660 TTCTCATGCT AAGAGTACGA
1840 GAAGCCAGGC CTTCGGTCCG	1930 CGGAGGCCTC GCCTCCGGAG	2020 AACCCAGGCC TTGGGTCCGG	2110 CCCCAAAGGC GGGGTTTCCG	2200 AAATCTTGTG TTTAGAACAC	2290 AGAGTAGCCT TCTCATCGGA	2380 GAGAGTGACC CTCTCACTGG	2470 CAAGAACCAG GTICTTGGTC	2560 CAACTACAAG GTTGATGTTC	2650 GGGGAACGTC CCCCTTGCAG
1830 GTGTCTGCTG CACAGACGAC	1920 CCTCTTCACC GGAGAAGTGG	2010 AGGTGCCCCT TCCACGGGGA	2100 CCTAAGCCCA GGATTCGGGT	2190 TGCAGAGCCC ACGTCTCGGG	2270 2280 2290 2390 2390 2390 2310 2330 2330 2340 CAAGGGGGGA CAGGTACCAA CATGTCCGGA GCCACATGGA GTTCCGCGA TCTCATCGA GTTAGGTCC TGTGTGGTG ACCCATGGTT GTACAGGCT CGGTGTACCA	2370 CCTCTGCCCT GGAGACGGGA	2460 ATGAGCTGAC TACTCGACTG	2540 2550 AGCAATGGGC AGCCGGAGAA TCGTTACCCG TCGGCCTCTT	2640 GGTGGCAGCA CCACCGTCGT
1810 1870 1880 1880 1890 00CCAGCAGCAG GOAGGCGAGG GOAGGCGGG GOAGGGGG GATCCCGGCT ATGCAGCCC AGTCCAGGGC CCGGTCGTG CCTCCCTCG ATGCAGGCCC AGTCCAGGCC AGTCCCGGG AGGACGACG CATCCCGGCC ATGCTCGGGG AGGCCCG AGGCCCCG AGGCCCCG AGGCCCCG AGGCCCCG AGGCCCCG AGGCCCCG AGGCCCCG AGGCCCCG AGGCCCCGGGG AGGCCCCCGGGG AGGCCCCGGGG AGGCCCCCGGGG AGGCCCCGGGG AGGCCCCGGGG AGGCCCCCGGGG AGGCCCCGGGG AGGCCCCCGGGG AGGCCCCCGGGG AGGCCCCGGGG AGGCCCCGGGG AGGCCCCGGGG AGGCCCCCGGGG AGGCCCCGGGG AGGCCCCCGGGG AGGCCCCCGGGG AGGCCCCCCCC	1910 1910 1920 1930 1930 1940 1950 1960 1960 1970 1980 1960 1970 1970 1980 1960 1970 1980 1980 1980 1980 1980 1980 1980 198	1990 2060 2060 2070 CTCCAGGCC CTCCACACAA AGGGGCAGGT GCTGGGCTCA GACCTGCCAA GAGCCATATC GAGACCCGT AGGTGCCCCT AACCCAGGCC CTCCACACATATC GAGACCCGT CGAGACCCGT CGAGACCCGT CGAGACCCGT CGAGACCCGT CGAGACCATATAG	2080 2090 2100 2110 2120 2130 2140 2150 CGGAGGACC CTGCCCTCTC ACTCCCTCAG CTCGGACACC TTCTCTCCTC GCCCTCTG GACGGGACT GGATTCGGGT GGGGTTTCCG GTTTGAGAGG TGAGGGGAGTC GAGCCTCTGG AAGAGAGGG	2170 2180 2190 2200 2210 2220 2230 2240 GHAACTCCCA ATCITCICT TGCAGAGAGCC AAATCITGTG ACAAAACTCA CACTGCCCA GGAGAGCCAGC CATTGAGAG TAGAAGAGAG ACGICCGGG TTTAGAACAC TGITTIGAGI GTGTACGGG GGCACGGGTC CATTCGGTC	2270 CAAGGCGGGA GTTCCGCCCT	2350 2400 2410 2420 2420 2420 2420 2420 242	2440 2500 2450 2510 2520 2520 2520 2520 2520 2520 25		2620 2630 2630 2650 2650 2670 2670 2680 2680 2680 2700 2680 2690 2700 2700 2680 2690 2700 2700 2700 2700 2700 2700 2700 27
1810 GGCCAGCACA CCGGTCGTGT	1900 AGCAAGGCAG TCGTTCCGTC	1990 GCTCTGGGCA CGAGACCCGT	2080 CGGGAGGACC GCCCTCCTGG	2170 GTAACTCCCA CATTGAGGGT	2260 CCCTCCAGCT GGGAGGTCGA	2350 CAGAGGCCGG GTCTCCGGCC	2440 CACCCTGCCC GTGGGACGGG	2530 GGAGTGGGAG CCTCACCCTC	2620 GCTCACCGTG CGAGTGGCAC

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2790	2880	2970	3060	3150	3240	3330	3420	3510	3600
TGCTTGGCAC	ATGGTTCTTT	TCTGCAGGTG	AGCAGCACCT	TTCTGTGAGC	CTACCCCCAC	CCTGTGGAGG	CACCACACAC	GAACACTCCT	TCAGACAAAC
ACGAACCGTG	TACCAAGAAA	ACACGTCCAC	TCGTCGTGGA	AAGACACTCG	GATGGGGGTG	GGACACCTCC	GTGGTGTGTG	CTTGTGAGGA	AGTCTGTTTG
2780	2870	2960	3050	3140	3230	3320	3410	3500	3590
CGCACGAGGA	CGAGACTGTG	TGGCCCAGGC	CCCTCCCTCC	GACTGTCCTG	CCTCACCCAT	ACTCTCGGGC	GCCACACGGC	TCGCACACGT	GCTGACCTGC
GCGTGCTCCT	GCTCTGACAC	ACCGGGTCCG	GGGAGGGAGG	CTGACAGGAC	GGAGTGGGTA	TGAGAGCCCG	CGGTGTGCCG	AGCGTGTGCA	CGACTGGACG
2770	2860	2950	3040	3130	3220	3310 3320	3400	3490 3500	3580
CTCTCGCGGT	TGGCCCCTG	GTCCCCACAC	GCCAGCGTGG	CTCTGTAGGA	ACAGGCCCTC	GGGGACATGC ACTCTCGGGC	AGGTTGGCCG	AGCAAGGTCC TCGCACACGT	TYCTCCACAT
GAGAGCGCCA	ACCCGGGGAC	CAGGGGTGTG	CGGTCGCACC	GAGACATCCT	TGTCCGGGAG	CCCCTGTACG TGAGAGCCCG	TCCAACCGGC	TCGTTCCAGG AGCGTGTGCA	AAGAGGTGTA
2750 2760 GCAAGCCCCGGGGCCCCCGGGGCCCCCGGGGCCCCCGGGGCCCC	2850 AGCGCTGCCC TCGCGACGGG	2930 2940 2950 2960 2960 CAGGCAGGC TGTGCAGGTG CTCCGACAC TGGCCCAGGTG CTCCGTCCAGG TGGGGGTGA CAGGGGTGA CAGGGGTGA CAGGGGTCAG ACGGGTCCAG	3030 TGGGGGATTT ACCCCCTAAA	3120 3130 3140 CAGCCCTGC CTCTGTAGGA GACTGTCCTG GTCGGGGACG GAGACATCCT CTGACAGGAC	3210 3220 3230 3230 3240 GTGCGTAGGG ACAGGCCTC CCTCACCCAT CTACCCCAC CACGCATCCC TGTCCGGGAG GGAGTGGGGTA GATGGGGGTG	3300 AACCGACTCC TTGGCTGAGG	3390 CCCCGCACTG GGGGCGTGAC	3480 CCCAGACCAG GGGTCTGGTC	3570 TCTCGGCAGC AGAGCCGTCG
2750	AGCATGGAAA TAAAGCACCC AGCGCTGCCC TGGGCCCCTG CGAGACTGTG ATGGTTCTTT TCGTACCTTT ATTTCGTGGG TCGCGACGGG ACCCGGGGAC GCTCTGACAC TACCAAGAAA	2930	3020	3110	3200	3290	3380	3470	3560
GCAAGCCCCC		GAGGCAGAGC	CTCGGCAGGG	GACAGACACA	CCTAGTCCAT	ATGGGGACAC	GITCAACAAA	CTGCACAGCA	CCCACGAGCC
CGTTCGGGGG		CTCCGTCTCG	GAGCCGTCCC	CTGTCTGTGT	GGATCAGGTA	TACCCCTGTG	CAAGITGITT	GACGTGTCGT	GGGTGCTCGG
2740	2830	2920	3010	3100	3190	3280	3370	3460	3550
GCGACGGCCG	AGCATGGAAA	TGGCATGAGG	AGGGGCTGCC	AGCCCCTGGQ	CGGGGGCATG	TCGCACCCGC	GCCCAGACCC	CCCGGGCGAA	CACCTCAAGG
CGCTGCCGGC	TCGTACCTTT	ACCGTACTCC	TCCCCGACGG	TCGGGGACCC	GCCCCCGTAC	AGCGTGGGCG	CGGGTCTGGG	GGGCCCGCTT	GTGGAGTTCC
2710 2710 2710 2710 2710 2710 2710 2710	2810 2820 2820 CARCCCCTG TACATACTTC CCGGGGCGCCC CATGGGGGAC ATGTATGAAG GGCCGCGGGG	2830 2940 2950 2960 2960 CCACGGGTCCACT GCCCCACAC TGGCCAGGC TGTGCAGGTG GGTCCCACT GCCCCACAC TGGCCAGGC TGTGCAGGTG GGTGCCCAGT GCCCCACAC TGGCGCTCAGG TGTGCAGGTG GGTGCCCAGT CCGGCTCAGA CTCGGGACTC ACCGTACTCC CTCCGTCTCCAC CCCAGGGTGA CAGGGGTCG ACACGTCCAC	1950 1950 1950 1960 1970 1970 1970 1970 1970 1970 1970 197	3070 3080 3080 3140 3150 3120 3120 3120 3130 3140 3150 3130 3140 3150 3140 3150 3140 3150 3150 3140 3150 3150 3150 3150 3150 3150 3150 315	3140 3220 3230 3230 3240 3240 3240 3240 32	3250 3300 3310 3310 3310 3310 3320 3300 3310 331	3340 3350 3350 3400 3400 3400 3390 3390 3400 3400 340	3430 3490 3500 3510 3450 3450 3470 3480 3490 3500 3500 3510 3500 3510 3500 3510 351	3520 3570 3580 3590 3590 3590 3590 3590 3590 3590 359
2720	2800 2810	2900	2990	3080	3170	3260	3350	3440	3530
CTGTCTCCGG	STACCCCTG TACATACTTC	GGCCGAGTCT	CCCTAGGGTG	GGGCCACGGG	TCCCGACCTC	CCTGGCTGCC	GATGCCCACA	GCCTCACACA	CCCCCACGAG
GACAGAGGCC	CATGGGGGAC ATGTATGAAG	CCGGCTCAGA	GGGATCCCAC	CCCGGTGCCC	AGGCTGGAG	GGACCGACGG	CTACGGGTGT	CGGAGTGTGT	GGGGGTGCTC
2710	2800	2890	2980	3070	3160	3250	3340	3430	3520
GAGCCTCTCC	GTACCCCCTG	CCACGGGTCA	TGCCTGGGCC	GCCCTGGGCT	GCCCTGTCC	GCCACTAACC	GACTGGTGCA	ACACGTGCAC	CGGACACAGG
CTCGGAGAGG	CATGGGGGAC	GGTGCCCAGT	ACGGACCCGG	CGGGACCCGA	CGGGGACAGG	CCGTGATTGG	CTGACCACGT	TGTGCACGTG	GCCTGTGTCC

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3690	3780	3870	3960	4050	4140	4230	4320	4400 4410	4500
TGGCCCACTT	CCCGTGCCTT	CATTCTATTC	ATGCCTTCTG	GTTACGCGCA	CCTCTCAAAA	CCCAGTTCCG	AAGTAGTGAG	TTGACGGCAA TCCTAGCGTG	ATTGGCAAGA
ACCGGGTGAA	GGGCACGGAA	GTAAGATAAG	TACCGAAGAC	CAATGCGCGT	GGAGAGTTTT	GGGTCAAGGC	TTCATCACTC	AACTGCCGTT AGGATCGCAC	TAACCGTTCT
3680	3770	3850 3860 3810	3940 3950	4040	4130	4220	4310		4490
TCCCTGGCCC	TTGCCCCTCC	CGCATTGTCT GAGTAGGTGT CATTCTATTC	CTGGGGATGC GGTGGGCTCT	GGGTGTGGTG	GTTCGCCGGG	CCTAACTCCG	GCTATTCCAG		AAATATGGGG
AGGGACCGGG	AACGGGGAGG	GCGTAACAGA CTCATCCACA GTAAGATAAG	GACCCCTACG CCACCCGAGA	CCCACACCCAC	CAAGCGGCCC	GGATTGAGGC	CGATAAGGTC		TTTATACCCC
3670	3760 3770	3850	3940 3950	4030	TTCCCTTCCT TTCTCGCCAC GTTCGCCGGG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCC	4200 4210 4220	4300	4180 4190	4480
CCACGTCACG	CATCTGTTGT TTGCCCTCC	CGCATTGTCT	CTGGGGATGC GGTGGGCTCT	TAAGCGCGGC		CTAACTCCGC CCATCCGGC CCTAACTCCG	CGGCCTCTGA	GCTGCGATTT CGCGCCAAAC	CCGTGTCCCA
GGTGCAGTGC	GTAGACAACA AACGGGGAGG	GCGTAACAGA	GACCCCTACG CCACCCGAGA	ATTCGCGCCG		GATTGAGGCG GGATTGAGGC	GCCGGAGACT	CGACGCTAAA GCGCGGTTTG	GGCACAGGGT
360 369 3670 3680 3690 GGATCACACA CCACACATT CCTAGACC ACGGGGTGAA	3750 AGTTGCCAGC TCAACGGTCG	3840 3850 3860 3870 GAAATTGCAT GGCATTGTCT GAGTAGGTGT CATTCTATTC CTTTAACGTA GCGTAACAGA CTCATCCACA GTAAGATAAG		4020 AGCGGCGCAT TCGCCGCGTA	4110 4120 4130 TTCCCTTCCT TTCTCGCCAC GTTCGCCGGG AAGAGCAAAGA AAGAGCGGTG CAAGCGGCCC	4200 CTAACTCCGC GATTGAGGCG	4390 4390 4300 4310 CAGCCGCGG CCCCGCGGGGGGGGGGGGGGGGGGGGGG	4380 GCTGCGATTT CGACGCTAAA	4470 TGCATCGTCG ACGTAGCAGC
3650 CACACACAGG GTGTGTGTCC	3730 3740 CAGCCTCGAC TGTGCCTTCT GTCGGAGCTG ACACGGAAGA	3830 ATAAAATGAG TATTTTACTC	3920 GGAAGACAAT CCTTCTGTTA	4010 CGCGCCCTGT GCGCGGGACA	4100 TTTCGCTTTC AAAGCGAAAG	4190 AGTCCCGCCC TCAGGGCGGG	4280 TGCAGAGGCC ACGTCTCCGG	AAAAGCTTGG ACAGCTCAGG TTTTCGAACC TGTCGAGTCC	4460 ACCATTGAAC TGGTAACTTG
CAGCCCTCC TCTCACAAGG GTGCCCTGC AGCCGCCACA CACACACAGG GCTCGGGAGG AGAGTGTTCC CACGGGGACG TCGGCGGTGT GTGTGTGTCC		3810 3810 3820 3820 3830 CGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG CCTTCCACGG TGAGGGTGAC AGGAAAGGAT TATTTTACTC	GGTGGGGCAG GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG CCACCCCGTC CTGTCGTTCC CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC	3980 3990 4000 4000 4010 4050 ACCORDER ADDRESSER ADDRESS	4050 4050 4090 4100 GCGTGACCC TACACTTTCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC CGCACTTGCC ATGTGAACG TCGCGGATC GCGGGCGAGG AAAGCGAAAG	AAGCATGCAT CTCAATTAGT CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG TCGTACGTG GATTAAATCA GTCGTTCGTA TCAGGGCGG GATTGAGGCG GGTAGGGCGG GGATTGAAGCC	4270 TTTTTATTTA AAAAATAAAT		4450 4450 4500 4500 450 4460 4470 4470 4480 4490 4500 4500 4500 4500 4500 4500 450
3630	3720	3810	3900	3990	4080	4150 4150 4160 4170	4260	4350	4440
Greccerec	CAGGACGGAT	ACTCCCACTG	GOTGGGGCAG GACAGCAAGG	GGCTCTAGGG	AGCGCCCTAG	AAGGGAAAAA AAGCATGCAT CTCAATTAGT	TGACTAATTT	TOGAGGCCTA GGCTTTTGCA	CCCGCTGCCA
CACGGGGACG	GTCCTGCCTA	TGAGGGTGAC	CCACCCGTC CTGTGGTTCC	CCGAGATCCC	TCGCGGGATC	TTCCCTTTTT CTCGTACGTA GAGTTAATCA	ACTGATTAAA	ACCTCCGGAT CCGAAAAGGT	GGGCGACGGT
3620	3710	3790 3800	3890	3980	4070	4160	4240 4250	4340	4430
TCTCACAAGG	CCCTTCCCTG	CCTTGACCCT GGAAGGTGCC	GGTGGGGCAG	AACCAGCTGG	TACACTTGCC	AAGCATGCAT	CCCATTCTCC GCCCCATGGC	TGGAGGCCTA	GGATTTTATC
AGAGTGTTCC	GGGAAGGGAC	GGAACTGGGA CCTTCCACGG	CCACCCGTC	TTGGTCGACC	ATGTGAACGG	TTCGTACGTA	GGGTAAGAGG CGGGGTACCG	ACCTCCGGAT	CCTAAAATAG
3610	3700	3790	3880	3970	4060	4150	4240	4330	4420
CCAGCCCTCC	CCCAGTGCCG	CCTTGACCCT	TGGGGGTGG	AGGCGGAAAG	GCGTGACCGC	AAGGGAAAA	CCCATTCTCC	GAGGCTYTTT	AAGGCTGGTA
GGTCGGGAGG	GGGTCACGGC	GGAACTGGGA	ACCCCCACC	TCCGCCTTTC	CGCACTGGCG	TTCCCTTTTT	GGGTAAGAGG	CTCCGAAAAA	TTCCGACCAT

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4590	4680	477	4860	4950	5040	5130	5220	531	5400
AAACAGAATC	AGTAGAGAAC	GCAAGTAAA	ACAAGGATCA	CTCTCTGAGG	GCTCCCTCC	TGACATAATT	TAATTGTTTG	CAGAAGAAA	AGGACTTTCC
TITGTCTIAG	TCATCTCTTG	CGTTCATTT	TGTTCCTAGT	GAGAGACTCC	CGAGGGGAGG	ACTGTATTAA	ATTAACAAAC	GTCTTCTTT	TCCTGAAAGG
4580 AGTGGAAGGT TCACCTTCCA	4600 4610 4620 4630 4630 4640 4650 4650 4660 4670 4680 4680 4680 4690 4670 4680 4690 4690 4690 4690 4690 4690 4690 469	4710 4710 4710 4720 4730 4730 4730 4730 4730 A730 A730 A730 A730 A730 A730 A730 A	4850 4850 4850 4850 4850 4850 4850 4850	4950 4950 4950 4950 4950 4950 4950 4950	4960 5010 5020 5030 5030 5040 5040 5040 5040 5030 503	5050 5110 5120 5130 5050 5070 5080 5090 5100 5100 5100 5120 5130 5130 5050 5050 5130 5130 5130 513	5220 5220 5220 5220 5220 5220 5220 5220	5210 5280 5290 5300 5310 5310 5270 5280 5290 5300 5310 5310 5300 5310 5310 5310 531	5370 5380 5390 5400 CAAAAAAGAA GAGAAGGTA GAAGACCCCA AGGACTTTCC GTTTTTTTCTT CTCTTTCCAT CTTCTGGGGT TCCTGAAAGG
4550 4560 4570 GPACTICCAA AGAATGACCA CAACCICTIC CATGAAGGIT TCITACTGGI GITGGAGAAG	4660	4750	4840	4930	5020	5110	5200	5290	5380
	ACAGAATTAA	TTATTGAACA	GCCACCTTAG	TCCCAGAATA	AAGATGCTTT	GGAACCTTAC	ATGTGTTAAA	TGAGGAAAAC	GAGAAAGGTA
	TGTCTTAATT	AATAACTTGT	CGGTGGAATC	AGGGTCTTAT	TTCTACGAAA	CCTTGGAATG	TACACAATTT	ACTCCTTTTG	CTCTTTCCAT
4560	4650	4740	4830	4920	5010	5100	5190	5280	5370
AGAATGACCA	CCTTTAAAGG	GCCTTAAGAC	AATCAACCAG	TATAAACTTC	GACTAACAGG	TCTTTGTGAA	TAAGTGTATA	ATGCCTTTAA	CAAAAAAGAA
TCTTACTGGT	GGAAATTTCC	CGGAATTCTG	TTAGTTGGTC	ATATTTGAAG	CTGATTGTCC	AGAAACACTT	ATTCACATAT	TACGGAAATT	GITITITITICIT
4550	4640	4730	4820	4910	S000	5090	5180	5270	5360
GTACTTCCAA	GAAGAATCGA	TITIGGATGAT	GGAAGCCATG	TTTGGGGAAA	CGAGAAGAAA	GCTTTAGATC	ATAAAATTTT	CAGTGGTGGA	TCTACTCCTC
CATGAAGGTT	CTTCTTAGCT	AAACCTACTA	CCTTCGGTAC	AAACCCCTTT	GCTCTTCTTT	CGAAATCTAG	TATTTTAAAA	GTCACCACCT	AGATGAGGAG
4540	4630	4720	4810 4820	4900	4990	5080	5170	5240 5250. 5250 5270 ATTCCAACCT ATGGAACTGA TGAATGGGAG CAGTGGTGGA TAAGGTTGGA TACCTTGACT ACTTACCCTC GTCACCACCT	5350
ACGAGTTCAA	CCATTCCTGA	TTGCCAAAAG	CTGTTTACCA GGAAGCCATG	CAGAAATIGA	TTGAAGTCTA	ACTTTTTGCTG	TAAGGTAAAT		CTCTCAACAT
TGCTCAAGTT	GGTAAGGACT	AACGGTTTTC	GACAAATGGT CCTTCGGTAC	GTCTFFAACT	AACTTCAGAT	TGAAAACGAC	ATTCCATTTA		GAGAGTTGTA
4530	4620	4710	4800	4890	4980	5070	5160	5250.	5340
CCGCTCAGGA	ACCTGGTTCT	GCTCATTITC	GGAGGCAGTT	ACGTTTTTCC	AAGTATAAGT	AGACCATGGG	TTTAAAGCTC	ATGGAACTGA	CTACTGCTGA
GGCGAGTCCT	TGGACCAAGA	CGAGTAAAAG	CCTCCGTCAA	TGCAAAAGG	TTCATATTCA	TCTGGTACCC	AAATTTCGAG	TACCTTGACT	GATGACGACT
4510 4520 4530 4540 ACGGAGACCT ACCCTGGCCT CGCTCAGGA ACGAGTTCAA TGCCTCTGGA TGGGACCGGA GGCGAGTCCT TGCTCAAGTT	4610 4600 4610 4620 4630 4630 16640 16GTGATTAT GGGTAGGAAA ACCTGGTTCT CCATTCCTGA GAAGAATCGA ACCTGGTTCT TGGACCAAGA GGTAAGGACT CTTCTTAGCT	4690 4700 4710 4710 TCANAGAACC ACCACGAGGA GCTCATTTTC AGTTCTTCT CGAGTAAAAG	4800 4790 4800 TAGATAGEC GAGGCAGTT ARCYCTACCA COTCCGTCAA	4880 TGAAAGTGAC ACTTTCACTG	4950 4970 4970 4990 5000 4990 5000 4090 TCCAGGAGGA AAAAGGCATC AAGTATAAGT TTGAAGTCTA CGAGAAGAAA AGGTCCTCCT TTTTCCGTAG TTCATATTCA AACTTCAGAT GCTCTTCTTT	5060 CATTITIATA GTAAAAATAT	5140 5180 5150 5200 GACHARACTR TARABATTIT TARGICIATA ATGIGITARA ATGIGITARA ATGIGITARA ATTICAGAGA TITARAGCIC TARGGIARAT ATARARITITARA ATTICACATITA TAITITARAA ATTICACATIT	5240 ATTCCAACCT TAAGGTTGGA	5320 5340 5340 5350 5360 GCCATCTAACAT TCTACTCCTC GGCAACATCT CATACTACTC GATGACGACT GAGAGTTGTA AGATGAGGAG
4510 ACGGAGACCT TGCCTCTGGA	4600 TGGTGATTAT ACCACTAATA	4690 TCAAAGAACC AGTITCTIGG	4780 TAGACATGGT ATCTGTACCA	4870 TCCAGGAATT ACGTCCTTAA	4960 TCCAGGAGGA	5050 TAAAGCTATG	S140 GGACAAACTA CCTGTTTGAT	5230 TGTATTTTAG ACATAAAATC	5320 GCCATCTAGT CGGTAGATCA

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5490	5580	S670	5760	5850	5940	6030	6120	6210	6300
AAAAAGCTGC	TTTTTCTTAC	TTAATAAGGA	CTCCCACACC	AGCAATAGCA	GTCTGGATCG	TACAAATAAA	TCTTATCATG	ACAATTCCAC	TCACTGCCCG
TTTTTCGACG	AAAAGAATG	AATTATTCCT	GAGGGTGTGG	TCGTTATCGT	CAGACCTAGC	ATGTTTATTT	AGAATAGTAC	TGTTAAGGTG	AGTGACGGGC
5480	5570	5660	5750	5840	5930	6020	6110	6200	6290
ACCACAAAGG	AACATACTGT	TCTAAAGGGG	TYTAAAAAAC	TTACAAATAA	ATCTTATCAT	TTATAATGGT	CATCAATGTA	TTATCCGCTC	TGCGTTGCGC
TGGTGTTTCC	TTGTATGACA	ACATTTCCCC	AAATYYYYYG	AATGTTTATT	TAGAATAGTA	AATATTACCA	GTAGTTACAT	AATAGGCGAG	ACGCAACGCG
5470	5560	5650	5740	S830	5920	6010	6100	6190	6280
TGCTATTTAC	TTATAATCAT	CTTTTTAATT	TTTTACTTGC	CTTATAATGG	TCATCAATGT	TTATTGCAGC	TGTCCAAACT	TGTGAAATTG	TCACATTAAT
ACGATAAATG	AATATTAGTA	GAAAAATTAA	AAAATGAACG	GAATATTACC	AGTAGTTACA	AATAACGTCG	ACAGGTTTGA	ACACTTTAAC	AGTGTAATTA
5460	5550	5640	5730	5820	5910	6000	6090	6180	6270
TTGCTTGCTT	GGCATAACAG	GTACCTTTAG	TTTGTAGAGG	TITATTGCAG	TTGTCCAAAC	CCCAACTTGT	AGTTGTGGTT	CTGTTTCCTG	GTCAGCTAAC
AACGAACGAA	CCGTATTGTC	CATGGAAATC	AAACATCTCC	AAATAACGTC	AACAGGTTTG	GGGTTGAACA	TCAACACCAA	GACAAAGGAC	CACTCGATTG
5450	5540	5630	5720	5810	5900	5990	6080	6170	6260
AATAGAACTC	TTTATAAGTA	CAAAAATTGT	CCATACCACA	TGTTAACTTG	TAGTTGTGGT	CTTCGCCCAC	ACTGCATTCT	ATGGTCATAG	TGCCTAATGA
TTATCTYGAG	AAATATTCAT	GTTTTTAACA	GGTATGGTGT	ACAATTGAAC	ATCAACACCA	GAAGCGGGTG	TGACGTAAGA	TACCAGTATC	ACGGATTACT
5410 5420 5430 5430 5440 5460 5460 5460 5460 5480 5480 5480 5480 5480 5480 5480 548	5500 5510 5520 5530 5540 5540 5550 5550 5570 5580 5560 5500 5500 5500 5500 5500 550	5590 5600 5600 5600 5620 5630 5630 5640 5650 5660 5600 TCACACACACAGG CATATTAATT TGTAAAGGGG TTAATAAGGA AGGIGTGTCC CATATTAACTA GACGATAATT ATTGATACGA GTTTTTAACA CATGGAAATT GAAAAATTCCT	5680 5780 5730 5730 5730 5730 5740 5750 5740 5750 5750 5740 5750 575	5770 5830 5840 5850 5850 5850 5850 5820 5820 5830 5830 5850 5850 5850 5850 5850 585	5910 5920 5930 5940 5940 5910 5920 5930 5940 1CACAAATTT CACTACTATIC TAGTICINGGT TIGICCAAAC TCATCAATGT ATCTTATCAT GTCTGGATCG AGGICTTAAA GTGTTTATTT CGTAAAAAA GTGACGTAAG ATCAACACCA AACAGGTTTG AGTAGTTACA TAGAATAGTA CAGACCTAGC	5950 6000 6010 6020 6030 6030 6030 6030 6030 6030 603	6040 6050 6050 6060 6070 6080 6080 6090 6100 6110 6120 GCAATAGCAT CACAAAȚITC ACAAATAAAG CATTITITIC ACTGCATICT AGTIGIGGIT TGTCCAAACT CATCAATGTA TCTTATCATG CGȚTATCGTA GTGTITAAAG TGTITATITC GTAAAAAAAG TGACGTAAGA TCAACACCAA ACAGGTITGA GTAGTTACAT AGAATAGTAC	6130 6140 6150 6160 6170 6180 6180 6200 6200 6200 6200 6200 6200 6200 62	6220 6250 6250 6250 6260 6270 6280 6280 6290 6300 ACAACATACS AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC TCACATTAAT TGCGTTGCG TCACTGCCCG TGTTGTATGC TCGGCCTTCG TATTTCACAT TTCGGACCCC ACGGATTACT CACTCGATTG AGTGTAATTA AGGCAACGCG AGTGACGGGC
5430	5520	5610	5700	5790	5880	5970	6060	6150	6240
TGAGTCATGC	TGGAAAATA	CTGCTATTAA	TGACTAGAGA	AAAATGAATG	GCATITITIT	GGGGATCTCA	ACAAATAAAG	AGCTAGAGCT	ATAAAGTGTA
ACTCAGTACG	ACCTITTIAT	GACGATAATT	ACTGATCTCT	TTTTACTTAC	CGTAAAAAAA	CCCCTAGAGT	TGTTTATTTC	TCGATCTCGA	TATTTCACAT
5420	5510	5600	5690	5780	5860	5960	6050	6140	6230
CTAAGTTTTT	AAGAAAATFA	CATAGAGTGT	TATAGTGCCT	CCTGAAACAT	TCACAATTT CACAATAAA	COTCCAGGGC	CACAAAITIC	GTCGACCTCT	AGCCGGAAGC
GATTCAAAAA	TTCTTTTAAT	GTATCTCACA	ATATCACGGA	GGACTTTGTA	AGTGTTTAAA GTGTTTATTT	GGAGGTCGCG	GTGTTTAAAG	CAGCTGGAGA	TCGGCCTTCG
5410	5500	5590	5680	5770	5860	5950	6040	6130	6220
TTCAGAATTG	ACTGCTATAC	TCCACACAGO	ATATTTGATG	TCCCCCTGAA	TCACAAATTT	GCTGGATGAT	GCAATAGCAT	TCTGTATACC	ACAACATACG
AAGTCTTAAC	TGACGATATG	AGGTGTGTCC	TATAAACTAC	AGGGGGACTT	AGTGTTTAAA	CGACCTACTA	CGTTATCGTA	AGACATATGG	TGTTGTATGC

Figure 14 (continued)

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6390 CGCTCTTCCG GCGAGAAGGC	6480 TTATCCACAG AATAGGTGTC	6570 GCGTTTTTCC CGCAAAAAGG	6660 TACCAGGCGT ATGGTCCGCA	6750 GGAAGCGTGG CCTTCGCACC	6840 CCCGTTCAGC GGGCAAGTCG	6930 ACTGGTAACA TGACCATTGT	7020 GTATTTGGTA CATAAACCAT	7110 GGTGGTTTTT CCACCAAAA	7200 7120 7130 7140 7150 7160 7170 7120 7120 7120 7120 7120 7130 7130 7130 7130 7130 7130 7130 713
6310 6320 6330 6340 6350 6350 6350 6370 6380 6390 6390 6390 6310 6390 6390 6390 6390 6390 6390 6390 639	6410 6410 6420 6420 6430 6430 6430 6440 6440 6440 6440 644	6510 6530 6540 6550 6520 6530 6530 6540 6550 6550 6540 6550 6550 6570 6570 6570 6570 6570 657	6650 6630 6640 6650 6650 6660 6620 6620 6630 6630 6640 6650 6660 6660 6660 6660 6660 666	6750 6730 6740 6750 6750 6750 6750 6750 6750 6750 675	6810 6810 6810 6810 6810 6810 6810 6810	6930 6930 6930 6930 6930 6930 6930 6930	1020 6980 7000 7000 7010 7020 6980 6980 8980 7000 7000 7010 7020 6980 6980 7000 7010 7020 7020 7020 7020 7020 702	7010 7030 7040 7050 7060 7060 7070 7030 7030 7030 7030 703	7190 GTCTGACGCT CAGACTGCGA
6370 GAGGCGGTTT CTCCGCCAAA	6460 ACTCAAAGGC TGAGTTTCCG	6550 GTAAAAAGGC CATTTERCCG	6640 ACCCGACAGG TGGGCTGTCC	6730 TGTCCGCCTT ACAGGCGGAA	6820 TGGGCTGTGT ACCCGACACA	6910 TATCGCCACT ATAGCGGTGA	7000 ACGGCTACAC TGCCGATGTG	7090 AACAAACCAC TYGTYTGGTG	7180 TTTCTACGGG AAAGATGCCC
6360 CGCGCGGGGA GCGCGCCCT	6450 GTATCAGCTC CATAGTCGAG	6540 GCCAGGAACC CGGTCCTTGG	6630 AGGTGGCGAA TCCACCGCTT	6720 ACCGGATACC TGGCCTATGG	6810 CGCTCCAAGC GCGAGGTTCG	6900 AGACACGACT TCTGTGCTGA	6990 TGGCCTAACT ACCGGATTGA	7080 TGATCCGGCA ACTAGGCCGT	7170 CCTTTGATCT GGAAACTAGA
6350 AATCGGCCAA TTAGCCGGTT	6440 GCGGCGAGCG CGCCGCTCGC	6530 CCAGCAAAAG GGTCGTTFTTC	6620 CTCAAGTCAG GAGTTCAGTC	6710 CCTGCCGCTT GGACGCCGAA	6800 GTAGGTCGTT CATCCAGCAA	6890 CAACCCGGTA GTTGGGCCAT	6980 CTTGAAGTGG GAACTTCACC	1010 TGGTAGCTCT ACCATCGAGA	7160 TCAAGAAGAT AGTTCTTCTA
6340 TGCATTAATG ACGTAATTAC	6430 TCGTTCGGCT AGCAAGCCGA	6520 GAGCAAAAGG CTCGTTTTCC	6610 AAAATCGACG TYTYTAGCYGC	6700 CTGTTCCGAC GACAAGGCTG	6790 TCAGTTCGGT AGTCAAGCCA	6880 GTCTTGAGTC CAGAACTCAG	.6970 CTACAGAGIT GATGTCTCAA	7060 GAAAAAGAGT CTTTTTTCTCA	7150 AAAAAGGATC TTTTTCCTAG
6330 TCGTGCCAGC AGCACGGTCG	6420 CTGCGCTCGG GACGCGAGCC	6540 6520 6530 6540 6520 6530 6540 6520 6540 6540 6540 6540 6540 6540 6540 654	6600 GAGCATCACA CTCGTAGTGT	6690 6690 6690 TTCCCCCTC AACTCCCTC GTCCCTCTC TTCCCCTCTC TTCCAGGGAG CACCGCAGAG	6780 TGTAGGTATC ACATCCATAG	6870 GGTAACTATC CCATTGATAG	6960 GTAGGCGGTG CATCCGCCAC	7030 7040 7050 7050 7050 7050 7050 7050 705	7140 ACCCCCAGAA TCCCCCTT
6310 6320 CTTTCCAGTC GGGAAACCTG GAAAGGTCAG CCCTTTGGAC	6410 CACTGACTCG	6500 TAACGCAGGA	6590 CCCCCTGAC	6680 AAGCTCCCTC	6770 ATGCTCACGC	6860 CGCCTTATCC	6950 AGCGAGGTAT TCGCTCCATA	TO40 GCTGAAGCCA	7130 GCAGCAGATT CGTCGICTAA
6310 CTTTCCAGTC GAAAGGTCAG	6400 CTTCCTCGCT	6490 AATCAGGGGA	6580 ATAGGCTCCG	6670 TTCCCCCTG	6760 CGCTTTCTCA	6850 CCGACCGCTG	6940 GGATTAGCAG	7030 TCTGCGCTCT	7120 TTGTTTTGCAA

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7290	7380	7470	7560	7650	7740	7830	7920	8010	8100
TTTAAATCAA	TTTCGTTCAT	CCGCGAGACC	TCCGCCTCCA	ACAGGCATCG	TTGTGCAAAA	CTGCATAATT	CGGCGACCGA	TCTTCGGGGC	TTTACTTTCA
AAATTTAGTT	AAAGCAAGTA	GGCGCTCTGG	AGGCGGAGGT	TGTCCGTAGC	AACACGTTTT	GACGTATTAA	GCCGCTGGCT	AGAAGCCCCG	AAATGAAAGT
7270 7280 7280	7370	7460	7540 7550 7560	7640	7730	7820	7910	8000	8090
TITITAAATTA AAAATGAAGT TITAAAITCAA	GATCTGTCTA	TGCAATGATA	GAAGTGGTCC TGCAACTTTA TCCGCCTCCA	TGCCATTGCT	ATCCCCCATG	TATGGCAGCA	ATAGTGTATG	TGGAAAACGT	TTCAGCATCT
AAAATITAAT TITITACTICA AAAITTAGTT	CTAGACAGAT	ACGTTACTAT	CTTCACCAGG ACGTTGAAAT AGGCGGAGGT	ACGGTAACGA	TAGGGGGTAC	ATACCGTCGT	TATCACATAC	ACCTTTTGCA	AAGTCGTAGA
7270	7360	7450	7540	7630	7720	7810	7900	7990	8080
TITTAAATTA	CTATCTCAGC	GCCCCAGTGC	GAAGTGGTCC	GCAACGTTGT	GAGTTACATG	CACTCATGGT	CATTCTGAGA	TGCTCATCAT	CCAACTGATC
AAAATITAAT	GATAGAGTCG	CGGGGTCACG	CTTCACCAGG	CGTTGCAACA	CTCAATGTAC	GTGAGTACCA	GTAAGACTCT	ACGAGTAGTA	GGTTGACTAG
7260 ACCTAGATCC TGGATCTAGG	7350 AGTGAGGCAC TCACTCCGTG	7440 TTACCATCTG AATGGTAGAC	7530 GCCGAGCGCA CGGCTCGCGT	7620 AATAGTTTGC TTATCAAACG	7710 CGATCAAGGC GCTAGTTCCG	7800 GCAGTGTTAT CGTCACAATA	7890 TCAACCAAGT AGTTGGTTCA	ACATAGCAGA ACTITAAAAG TGCTCATCAT TGGAAAACGT TGTATCGTCT TGAAATTTTC ACGAGTAGTA ACCTITTTGCA	8060 8070 8080 8090 8100 GAIGTAACCC ACTCGIGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CTACATTGGG TGAGCACGTG GGTTGACTAG AAGTCGTAGA AAATGAAAGT
7250	7340	7430	7520	7610	7700	7790	7880	7970	8060
AAGGATCTTC	ATGCTTAATC	ACGGGAGGGC	AGCCGGAAGG	TTCGCCAGTT	CGGTTCCCAA	TAAGTTGGCC	TGGTGAGTAC	ACATAGCAGA	GATGTAACCC
TTCCTAGAAG	TACGAATTAG	TGCCCTCCCG	TCGGCCTTCC	AAGCGGTCAA	GCCAAGGGTT	ATTCAACCGG	ACCACTCATG	TGTATCGTCT	CTACATTGGG
7240 GATTATCAAA CTAATAGTTT	7330 ACAGTTACCA TGTCAATGGT	970 7410 7420 7430 7440 7450 7450 7460 7460 7470 GCCCCAGTGC TGCAATGATA CCGCGAGACC CAGCACATCT ATTGATGCTA TGCCCTCCCG ANTGGTAGAC CGGGGTCACG ACGTTACTAT GGCGTCTGG	7510 TAAACCAGCC ATTTGGTCGG	7600 GAGTAAGTAG CTCATTCATC	7690 CATTCAGCTC GTAAGTCGAG	CCTCCGATCG TYCTCAGAAG TAACTYGGCC GCAGYGTTAT CACYCATGGT TAYGGCAGCA CYGCATAATY GGAGGCTAGC ACAGYCTTC ATYCAACCGG CGYCACAATA GYGAGGCTAGC ACAGYCTTC ATYCAACCGG CGYCACAATA GYGAGGTACCA ATACCGTCGT GACGYATTAA	7870 TITICIGIGAC AAAGACACTG	ATACGGGATA ATACCGCGCC TATGCCCTAT TATGCCGGG	8050 GATCCAGTTC CTAGGTCAAG
7210 7220 7230 7250 7250 7250 7250 7250 7250 7250 725	7380 7380 7370 7380 7380 7380 7380 7380	7330 7450 7460 7460 7470 CCATAGITICS CONTROCAL ACCICAGICS TINCCATCITS GCCCCAGIGS TGCAATGATA CCGCGAGACC GGTATCATA ACTAGATA ACCCTACCT TINCCATCITS GCCCCAGIGS TGCTATCATA GCCCTAGAGGC CAGCACATCT ATTGATGCTA TGCCCTCCCG ANTGGTAGAG CGGGTCACG ACGTTACTAT GGCGTTCTGG	7500 7510 7510 7520 7520 7520 7520 7550 7550 7550 755	7570 7580 7580 7640 7650 7610 7620 7620 7630 7630 7640 7650 7650 7650 7650 7640 7650 7650 7650 7650 7650 7650 7650 765	730 7720 7720 7730 7730 7730 7730 7730 7	CCTCCGATCG TYGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT GCAGGCTAGC AACAGTCTTC ATTCAACCG CGTCACAATA GTGAGTACCA ATACCGTCGT GACGTATAA	7810 7850 7850 7850 7850 7850 7850 7850 785	7930 7950 7950 7960 7970 7970 7980 7990 8000 8010 GTTGCTCTTG CCCGGCGTCA ATACCGGGGC ACATAGCAGA ACTITAAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC CAACGAGAAC GGGCGGCAGT TATGCCCTAT TATGGCGCGG TCTATCGTCT TGAAATTTTC ACGAGTAGTA ACCTTTTGCA AGAAGCCCCG	8020 8030 8040 8050 8060 8070 8050 8060 8070 8080 8090 8100 GAAAACTICTIC AAGGATCTTTA CCGCTGTAGA GATCCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CTTTTGAGAG TTCCTAGAAT GGCGACAACT CTAGGTCAAG CTACATTGAGA GGTTGACTAG AAGTCGTAGA AAATGAAAGT
7220	7310	7400	7490	7580	7670	7760	7850	0140	8030
TTAAGGGATT	ATATGAGTAA	CTGACTCCCC	GGCTCCAGAT	TAATTGTTGC	CTCGTCGTTT	CTCCTTCGGT	CATGCCATCC	GITGCTCTTG CCCGCGTCA	AAGGATCTTA
AATTCCCTAA	TATACTCATT	GACTGAGGGG	CCGAGGTCTA	ATTAACAACG	GAGCAGCAAA	GAGGAAGCCA	GTACGGTAGG	CAACGAGAAC GGGCGCAGT	TTCCTAGAAT
7210	7300	7390	7480	7570	7660	7750	7840	7930	8020
AAAACTCACG	TCTAAAGTAT	CCATAGTTGC	CACGCTCACC	TCCAGTCTAT	TGGTGTCACG	AAGCGGTTAG	CTCTTACTGT	GITGCTCTTG	GAAAACTCTC
TTTTGAGTGC	AGATTTCATA	GGTATCAACG	GTGCGAGTGG	AGGTCAGATA	ACCACAGTGC	TTCGCCAATC	GAGAATGACA	CAACGAGAAC	CTTTTGAGAG

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8190 CTCATACTCT GAGTATGAGA	8280 AAACAAATAG TTTGTTTATC	
8180 ATGTTGAATA TACAACTTAT	8270 TTAGAAAAT AATCTTTTTA	
8170 CGACACGGAA GCTGTGCCTT	8260 TTGAATGTAT AACTTACATA	
8160 GGAATAAGGG CCTTATTCCC	8250 GGATACATAT CCTATGTATA	
8150 CGCAAAAAG GCGTTTTTTC	8240 TCTCATGAGC AGAGTACTCG	8330 G
8110 8120 8130 8140 8150 8150 8160 8160 8170 8180 8180 8190 8190 8190 8190 8190 819	8280 8250 8210 8270 8270 8280 8280 8250 8250 8260 8270 8280 8280 8280 8270 8280 8280 8270 8280 828	8290 8300 8300 8310 8320 8320 CACTGACGT CCCAAGGCG CACATATCCC CGAAAAATGC CACCTGACGT CCCAAGGCG GCTTTTCACG GTGGACTGCA G
8130 AAAACAGGAA TYYYGTCCTT	8220 AGCATTTATC TCGTAAATAG	8310 CGAAAAGTGC GCTTTTCACG
8120 TGGGTGAGCA ACCCACTCGT	8210 ATATTATTGA TATAATAACT	8300 CACATTTCCC GTGTAAAGGG
8110 CCAGCGTTTC GGTCGCAAAG	8200 TCCTTTTTCA AGGAAAAGT	8290 GGGTTCCGCG CCCAAGGCGC

Comparison of whole chiBR96 and deleted CH2 chiBR96 on Ley/K ELISA

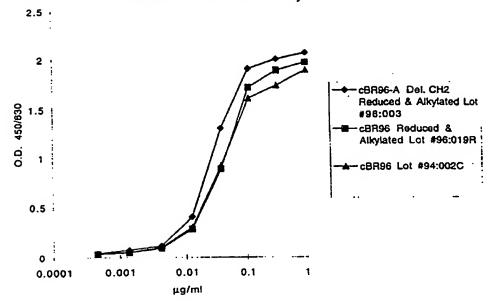


Figure 15

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hBR96-2B: L235 to A235 and G237 to A237

hBR96-2C: E318 to S318, K320 to S320, and K322 to S322

hBR96-2D: P331 to A331

hBR96-2E: L235 to A235, G237 to A237, E318 to S318, K320 to S320, and

K322 to S322

hBR96-2F: L235 to A235, G237 to A237, and P331 to A331

hBR96-2G: E318 to S318, K320 to S320, K322 to S322, and P331 to A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320, K322 to S322, and P331 to A331



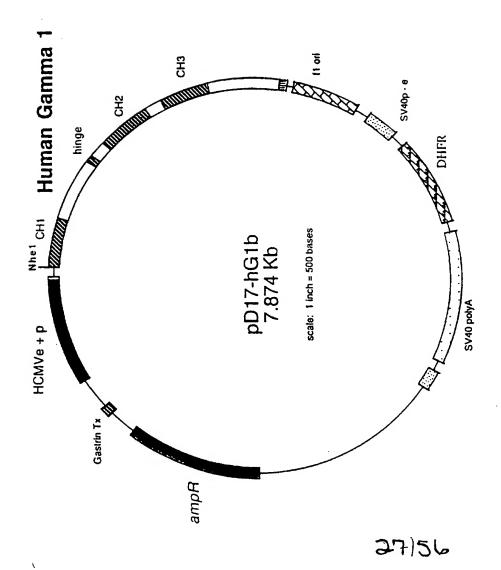


FIGURE 18A

1	GGTACCAATT	TAAATTGATA	TCTCCTTAGG	TCTCGAGTCT	CTAGATAACC
51	GGTCAATCGA	TTGGAATTCT	TGCGGCCGCT	TGCTAGCCAC	CATGGAGTTG
101	TGGTTAAGCT	TGGTCTTCCT	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA
151	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	AGTGCAGCCT	GGAGGGTCCC
201	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	CTATTACATG
251	TATTGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT
301	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT
351	TCACCATCTC	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC
401	AGCCTGAGGG	ACGAGGACAC	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC
451	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	AGGGACTCTG	GTCACGGTCT
501	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	ACCCTCCTCC
551	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA
601	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG
651	GCGTGCACAC	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC
701	AGCAGCGTGG	TCACCGTGCC	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT
751	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	CAAGGTGGAC	AAGAAAGTTG
801	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	CCAGGCTCAG
851	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA
901	AGGCAGGCCC	CGTCTGCCTC	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC
951	ATGCTCAGGG	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA
1001	CAGGCTAGGT	GCCCCTAACC	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG
1051	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	AGGACCCTGC	CCCTGACCTA
1101	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	GACACCTTCT
1151	CTCCTCCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAAT
1201	CTTGTGACAA	AACTCACACA	TGCCCACCGT	GCCCAGGTAA	GCCAGCCCAG
1251	GCCTCGCCCT	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT
1301	CCAGGGACAG	GCCCCAGCCG	GGTGCTGACA	CGTCCACCTC	CATCTCTTCC

		235	237		
1351	TCAGCACCTG	AACTOTTGG	GGGACCGTCA	GTCTTCCTCT	TCCCCCCAAA
1401	ACCCAAGGAC	ACCCTCATGA	TCTCCCGGAC	CCCTGAGGTC	ACATGCGTGG
1451	TGGTGGACGT	GAGCCACGAA	GACCCTGAGG	TCAAGTTCAA	CTGGTACGTG
1501	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG	AGGAGCAGTA
1551	CAACAGCACG	TACCGTGTGG		CACCGTCCTG	CACCAGGACT
1601		CAAGGAGTAC	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA
1651	GCCCCATCG	AGAAAACCAT	CTCCAAAGCC	AAAGGTGGGA	CCCGTGGGGT
1701	GCGAGGGCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCTC	TGCCCTGAGA
1751	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA
1801	GGTGTACACC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA
1851	GCCTGACCTG	CCTGGTCAAA	GĠCTTCTATC	CCAGCGACAT	CGCCGTGGAG
1901	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT
1951	GCTGGACTCC	GACGGCTCCT	TCTTCCTCTA	CAGCAAGCTC	ACCGTGGACA
2001	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG
2051	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA
2101	ATGAGTGCGA	CGGCCGGCAA	GCCCCCCCTC	CCCGGGCTCT	CGCGGTCGCA
2151	CGAGGATGCT	TGGCACGTAC	CCCCTGTACA	TACTTCCCGG	GCGCCCAGCA
2201	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	CCCCTGCGAG	ACTGTGATGG
2251	TTCTTTCCAC	GGGTCAGGCC	GAGTCTGAGG	CCTGAGTGGC	ATGAGGGAGG
2301	CAGAGCGGGT	CCCACTGTCC	CCACACTGGC	CCAGGCTGTG	CAGGTGTGCC
2351	TGGGCCCCCT	AGGGTGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG
2401	GGATTTGCCA	GCGTGGCCCT	CCCTCCAGCA	GCACCTGCCC	TGGGCTGGGC
2451	CACGGGAAGC	CCTAGGAGCC	CCTGGGGACA	GACACACAGC	CCCTGCCTCT
2501	GTAGGAGACT	GTCCTGTTCT	GTGAGCGCCC	CTGTCCTCCC	GACCTCCATG
2551	CCCACTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	GCCCTCCCTC
2601	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC
2551	ACCCGCATGG	GGACACAACC	GACTCCGGGG	ACATGCACTO	TCGGGCCCTG
2701	TGGAGGGACT	GGTGCAGATG	CCCACACACA	CACTCAGCCC	AGACCCGTTC
2751	AACAAACCCC	GCACTGAGGT	TGGCCGGCCA	CACGGCCACC	ACACACACAC
2801	GTGCACGCCT	CACACACGGA	GCCTCACCCG	GGCGAACTGC	ACAGCACCCA

2851	GACCAGAGCA	AGG + CCTCGC	ACACGTGAAC	ACTCCTCGGA	CACAGGCCCC
2901	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT
2951	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC
3001	CCCTGCAGCC	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC
3051	TGGCCCTGGC	CCACTTCCCA	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC
3101	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG
3151	TGCCTTCCTT	GACCCTGGAA	GGTGCCACTC	CCACTGTCCT	TTCCTAATAA
3201	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG
3251	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA
3301	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC
3351	AGCTGGGGCT	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAG
3401	; CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG
3451	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTCC	CTTCCTTTCT	CGCCACGTTC
3501	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAAGC	ATGCATCTCA	ATTAGTCAGC
3551	AACCATAGTC	CCGCCCTAA	CTCCGCCCAT	CCCGCCCCTA	ACTCCGCCCA
3601	GTTCCGCCCA	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA
3651	GAGGCCGAGG	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG
3701	CTTTTTTGGA	GGCCTAGGCT	TTTGCAAAAA	GCTTGGACAG	CTCAGGGCTG
3751	CGATTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	CTGGTAGGAT
3801	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCGCCGT
3851	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC
3901	TCAGGAACGA	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG
3951	GAAGGTAAAC	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT
4001	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	AATTAATATA	GTTCTCAGTA
4051	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	CAAAAGTTTG
4101	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA
4151	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC
4201	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA
4251	AGTGACACGT	TTTTCCCAGA	AATTGATTTG	GGGAAATATA	AACTTCTCCC
4301	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	GGAGGAAAA	GGCATCAAGT

4351	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGA <u>A</u> GA	TGCTTTCAAG
4401	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT
4451	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC
4501	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA
4551	AATTTTTAAG	TGTATAATGT	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA
4601	TTTTAGATTC	CAACCTATGG	AACTGATGAA	TGGGAGCAGT	GGTGGAATGC
4651	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	TCTAGTGATG
4701	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA
4751	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG
4801	TCATGCTGTG	TTTAGTAATA	GAACTCTTGC	TTGCTTTGCT	ATTTACACCA
4851	CAAAGGAAAA	AGCTGCACTG	CTATACAAGA	AAATTATGGA	AAAATATTCT
4901	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	AATCATAACA	TACTGTTTTT
4951	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	TATGCTCAAA
5001	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTTAA	TAAGGAATAT
5051	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG
5101	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG
5151	AAACATAAAA	TGAATGCAAT	TGTTGTTGTT	AACTTGTTTA	TTGCAGCTTA
5201	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	AAATTTCACA	AATAAAGCAT
5251	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	CAATGTATCT
5301	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT
5351	GGAGTTCTTC	GCCCACCCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA
5401	AATAAAGCAA	TAGCATCACA	AATTTCACAA	ATAAAGCATT	TTTTTCACTG
5451	CATTCTAGTT	GTGGTTTGTC	CAAACTCATC	AATGTATCTT	ATCATGTCTG
5501	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT
5551	TTCCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC
5601	GGAAGCATAA	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC
5651	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCGT
5701	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCGT
5751	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT
5801	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT

FIGURE 18D

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5351	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG
5901	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG
5951	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT
6001	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC
6051	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTC
6101	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	TTCTCAATGC	TCACGCTGTA
6151	GGTATCTCAG	TTCGGTGTAG	GTCGTTCGCT	CCAAGCTGGG	CTGTGTGCAC
6201	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT
6251	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG
6301	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG
6351	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG
6401	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT
6451	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG
6501	CAGATTACGC	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC
6551	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG
6601	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAA
6651	TGAAGTTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG
5701	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGCGATC	TGTCTATTTC
6751	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG	TGTAGATAAC	TACGATACGG
6801	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	GAGACCCACG
6851	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG
6901	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT
6951	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG	CCAGTTAATA	GTTTGCGCAA
7001	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	GTCACGCTCG	TCGTTTGGTA
7051	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	TACATGATCC
7101	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCGGTCCTC	CGATCGTTGT
7151	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC
7201	ATAATTCTCT	TACTGTCATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT
7251	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG
7301	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	CGCGCCACAT	AGCAGAACTT

7351	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT	CGGGGCGAAA	ACTCTCAAGG
7401	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCCAA
7451	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA
7501	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT
7551	TGAATACTCA	TACTCTTCCT	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG
7601	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA	ATGTATTTAG	AAAAATAAAC
7651	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	TGACGTCGAC
7701	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGGCT	TCGAATAGCC
7751	AGAGTAACCT	TTTTTTTAA	TTTTATTTTA	TTTTATTTTT	GAGATGGAGT
7801	TTGGCGCCGA	TCTCCCGATC	CCCTATGGTC	GACTCTCAGT	ACAATCTGCT
7851	CTGATGCCGC	ATAGTTAAGC	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG
7901	GTCGCTGAGT	AGTGCGCGAG	CAAAATTTAA	GCTACAACAA	GGCAAGGCTT
7951	GACCGACAAT	TGCATGAAGA	ATCTGCTTAG	GGTTAGGCGT	TTTGCGCTGC
8001	TTCGCGATGT	ACGGGCCAGA	TATACGCGTT	GACATTGATT	ATTGACTAGT
8051	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTCATAGCC	CATATATGGA
8101	GTTCCGCGTT	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA
8151	ACGACCCCCG	CCCATTGACG	TCAATAATGA	CGTATGTTCC	CATAGTAACG
8201	CCAATAGGGA	CTTTCCATTG	ACGTCAATGG	GTGGACTATT	TACGGTAAAC
8251	TGCCCACTTG	GCAGTACATC	AAGTGTATCA	TATGCCAAGT	ACGCCCCTA
8301	TTGACGTCAA	TGACGGTAAA	TGGCCCGCCT	GGCATTATGC	CCAGTACATG
8351	ACCTTATGGG	ACTTTCCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC
8401	TATTACCATG	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC
8451	GGTTTGACTC	ACGGGGATTT	CCAAGTCTCC	ACCCCATTGA	CGTCAATGGG
8501	AGTTTGTTTT	GGCACCAAAA	TCAACGGGAC	TTTCCAAAAT	GTCGTAACAA
8551	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG	GCGTGTACGG	TGGGAGGTCT
8601	ATATAAGCAG	AGCTCTCTGG	CTAACTAGAG	AACCCACTGC	TTACTGGCTT
8651	ATCGAAATTA	ATACGACTCA	CTATAGGGAG	ACCCAAGCTT	

FIGURE 18F

60	120	180	240	300	360	420	480	540	600
GGTCAATCGA	CCTGGCACCC	GGACTACTTC	GCACACCTTC	CGINGCCCTNCC	CAACACCAAG	TGGAAGCCAG	GCAGCAAGGC	TCAGGGAGAG	CTAACCCAGG
CCAGTTAGCT	GGACCGTGGG	CCTGAIGAAG	CGTGTGGAAG	GCACGGGAGG	GT'IGTGGT'IC	ACCTTCGGTC	CGTCGTTCCG	AGTCCCTCTC	GATTGGGTCC
50	110	170	230	290	350	410	470	530	590
CTAGATAACC	CGGTCTTCCC	GCCTGGTCAA	CCAGCGGCGT	GCGTGGTCAC	ACAAGCCCAG	GGGTGTCTGC	CCAGTCCAGG	CCACTCATGC	CTAGGTGCCC
GATCTATTGG	GCCAGAAGGG	CGGACCAGTT	GGTCGCCGCA	CGCACCAGTG	TGTTCGGGTC	CCCACAGACG	GGTCAGGTCC	GGTGAGTACG	GATCCACGG
40	100	160	220	280	340	400	460	520	580
TCTCGAGTCT	AAGGGCCCAT	GCCCTGGGCT	GGCGCCCTGA	TCCCTCAGCA	AACGTGAATC	CAGGGAGGGA	CTATGCAGCC	TCTGCCCGCC	CAGGCACAGG
AGAGCTCAGA	TTCCCGGGFA	CGGGACCCGA	CCGCGGGACT	AGGGAGTCGT	TTGCAC1TAG	GTCCCTCCCT	GATACGTCGG	AGACGGGCGG	GTCCGTGTCC
30	90	150	210	270	330	390	450	510	570
TCTCCTTAGG	TGCTAGCACC	GGGCACAGCG	GTGGAACTCA	AGGACTCTAC	CTACATCTGC	GAGGCCAGCA	CGCATCCCGG	CCCGGAGGCC	AGGCTCTGGG
AGAGGAATCC	ACGATCGTGG	CCCGTGTCGC	CACCTTGAGT	TCCTGAGATG	GATGTAGACG	CTCCGGTCGT	GCGTAGGGCC	GGGCCTCCGG	TCCGAGACCC
20	80	140	200	260	320	380	440	500	560
TAAATTGATA	TGCGGCCGCT	GCACCTCTGG	TGACGGTGTC	TACAGTCCTC	GCACCCAGAC	AAGTTGGTGA	CCTGCCTGGA	TGCCTCTTCA	CTTTTTCCCC
ATTTAACTAT	ACGCCGGCGA	CGTGGAGACC	ACTGCCACAG	ATGTCAGGAG	CGTGGGTCTG	!PTCAACCAC'F	GGACGBACCT	ACGGAGAAGT	GAAAAAGGGG
10 GGTACCAATT '	70 TYGGAAT'I'CT AACCTYFAAGA	130 TCCTCCAAGA AGGAGGTTCT	190 CCCGAACCGG GGGCTTGGCC	250 CCGGCTGTCC GGCCGACAGG	310 AGCAGCTTGG TCGTCGAACC	370 GTGGACAAGA CACCTGTTCT	430 GCTCAGCGCT CGAGTCGCGA	490 AGGCCCCGTC TCCGGGCAG	550 GGTCTTCTGG CCAGAAGACC

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FIGURE 19 A

660 TCCGGGAGGA AGGCCCTCCT	720 AGCTCGGACA TCGAGCCTGT	780 CCAAATCTTG GGTTTAGAAC	830 840 GCCCAGGCCT CGCCCTCCAG CGGGTCCGGA GCGGGAGGTC	890 900 GGACAGGCCC CAGCCGGGTG CCTGTCCGGG GTCGGCCCAC	235 950234 960 deresegga cesteagrer deacteces secasteasa	AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT 1"FCCTGTGGG AGIACTACAG GCCCTGGGGA CTCCAGTGTA	1070 1080 GTTCAACTGG TACGTGGACG CAAGTTGACC ATGCACCTGC	AGCACG! !rcg!rgc!	SAGITACAAGI CTCATGITTCA
650 AAGAGCCATA TTCTCGGTAT	710 CCACTCCCTC GGTGAGGGAG	770 ICTGCAGAGC AGACGTCTCG		890 GGACAGGCCC CCTGTCCGGG		1010 CCGGACCCCT GCCCTGGGGA		CGCGGGAGGA GCAGTACAAC GCGCCCTCC'! CGTCATGITYG	1180 1190 AGGACTGGCT GAATGGCAAG TCCTGACCGA CTTACGTTC
640 CAGACCTGCC GTCTGGACGG	700 GCCAAACTCT CGGTTTGAGA	760 CAATC/FTC/FC GTTAGAAGAG	820 AGGTAAGCCA TCCATTCGGT	880 CTGCATCCAG GACGTAGGTC	940 CACCTGAACT GTGGACTTGA	AAGGACACCC TCATGAICTC 1"FCCTGTGGG AGIACTAGAG	1060 CTGAGGTCAA GACTCCAGIT	1120 CGCGGGAGGA GCGCCCTCCT	1180 AGGACTGGCT TCCTGACCCA
630 640 650 GTGCTGGGCT CAGACCTGCC AAGAGCCATA CACGACCCGA GTCTGGACGG TTCTCGGTAT	690 CACCCCAAAG GTGGGGTTTC	750 CAGTAACTCC GTCATTGAGG	810 CACCGTGCCC GTGGCACGGG	870 CTAGAGTAGC GATCTCATCG	930 TCTTCCTCAG AGAAGGAGTC	990 AAGGACACCC 1"FCCTGTGGG	1050 CACGAAGACC GTGCTTCTGG	1110 AAGACAAAGC 1YICTGTYTGG	1170 GTCCTGCACC CAGGACGTGG
620 AAAGGGGCAG TPTCCCCGTC	680 GACCTAAGCC CTGGATTCGG	740 TCCCAGATTC AGGGTCTAAG		860 GACAGGTGCC CTGTCCACGG	920 CACCTCCATC GTGGAGGTAG	980 970 TCCTCTTCC CCCAAAACCC AGGAGAAGGG GGGTTTTTGGG	1040 GGACGTGAGC CCTGCACTCG		1150 1150 GTCTGCTCAG CGTCCTCACC CACACCAGTIC GCAGGAGTIGG
610 CCCTGCACAC GGGACCTGTG	670 680 CCCTGCCCT GACCTAAGCC GGGACGGGA CTGGATTCGG	730 CCTTCTCTCC GGAAGAGAGG			910 CTGACACGTC GACTGTGCAG			1090 CCGTGGAGGT	1150 CTCTGCTCAG CACACCACTC
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1260	1320	1380	1440	1500	1560	1620	1680	1740	1800
AAAGCCAAAG	ACCCTCTGCC	ACCACAGGTG	GACCTGCCTG	GCAGCCGGAG	CCTCTACAGC	CTCCGTGATG	GGGTAAATGA	GATGCTTGGC	CCAGCGCTGC
TTTCGGTTTC	TGGGAGACGG	TGGTGTCCAC	CTGGACGGAC	CGTCGGCCTC	GGAGATGTCG	GAGGCACTAC	CCCATTTACT	CTACGAACCG	GGTCGCGACG
1250	1310	1370 1380	1430	1490	1550	1610	1670	1730	1790
AACCATCTCC	GGCTCGGCCC	AGCCCCGAGA ACCACAGGTG	AGGTCAGCCT	AGAGCAATGG	GCTCCTTCTT	TCTTCTCATG	CCCTGTCTCC	GTCGCACGAG	AATAAAGCAC
TYGGTAGAGG	CCGAGCCGGG	TCGGGGCTCT TGGTGTCCAC	TCCAGTCGGA	TCTCGTTACC	CGAGGAAGAA	AGAAGAGTAC	GGGACAGAGG	CAGCGTGCTC	IITATIITCGTG
CTCCCAGCC CCATCGAGAA GAGGGTCGG GGFAGCTCTT	1300 GACAGAGGCC CTGTCTCCGG	1360 CCTACAGGGC GGATGTCCCG	1420 ACCAAGAACC TGGTTCTTGG	1480 GTGGAGTGGG CACCTCACCC	1540 GACTCCGACG CTGAGGCTGC	1600 CAGGGGAACG GTCCCCTTGC	1660 AAGAGCCTCT TTCTCGGAGA	GCTCTCGCG CCGAGAGCGC	CCAGCATGGA AATAAAGCAC GGTCGTACC'F 'PTATTTCGTG
123023	1290	1350	1410	1470	1530	1590	1650	1710	1770
CTCCCAGCCC	GGGCCACATG	AACCTCTGTC	GGATGAGCTG	CGACATCGCC	TCCCGTGCTG	CAGGTGGCAG	CTACACGCAG	CCGCTCCCCG	TCCCGGGCGC
CAGGGTCGGG	CCCGGTGTAC	TTGGAGACAG	CCTACTCGAC	GCTGTAGCGG	AGGGCACGAC	GTCCACCGTC	GATGTGCGTC	GGCGAGGGGC	AGGGCCCGGG
1220	1280	1340	1400	1460	1520	1580	1640	1700	1760
CAACAAAGCC	TGGGGTGCGA	CCGCTGTACC	CCCCATCCCG	TCTATCCCAG	AGACCACGCC	TGGACAAGAG	TGCACAACCA	CGGCAAGCCC	TGTACATACT
GTTGTTTCGG	ACCCCACGCT	GCCGACATGG	GGGGTAGGGC	AGATAGGGTC	TCTGGTGCGG	ACCTGTTCTC	ACGTGTTGGT	GCCGTTCGGG	ACATGTATGA
312 1210	1270	1330	1390	1450	1510	1570	1630	1690	1750
OCAAGGTCTC	GTGGGACCCG	CTGAGAGTGA	TACACCCTGC	GTCAAAGGCT	AACAACTACA	AAGCTCACCG	CATGAGGCTC	GTGCGACGGC	ACGTACCCCC
CSTTCCAGAG	CACCCTGGGC	GACTCTCACT	ATGTGGGACG	CAGTTTCCGA	TTGTTGATGT	TYCGAGTGGC	GTACTCCGAG	CACGCTGCCG	TCCATCCGGG

FICURE 19C

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1860	1920	1980	2040	2100	2160	2220	2280	2340	2400
CTGAGGCCTG	GCTGTGCAGG	GGTGGGGGAT	GGAAGCCCTA	TGTTCTGTGA	GCGGTGGGCT	CACGCGCCCT	GCTACACTTG	ACGTTCGCCG	AGTGCTTTAC
GACTCCGGAC	CGACACGTCC	CCACCCCTA	CCTTCGGGAT	ACAAGACACT	CGCCACCCGA	GTGCGCGGGA	CGATGTGAAC	TGCAAGCGGC	TCACGAAATG
1850	1910	1970	2030	2090	2150	2210	2270	2330	2390
CAGGCCGAGT	ACTGGCCCAG	CCCTCGGCAG	CTGGGCCACG	GAGACTGTCC	TGCTGGGGAT	GGGGTATCCC	CAGCGTGACC	CTTTCTCGCC	GTTCCGATTT
GTCCGGCTCA	TGACCGGGTC	GGGAGCCGTC	GACCCGGTGC	CTCTGACAGG	ACGACCCCTA	CCCCATAGGG	GTCGCACTGG	GAAAGAGCGG	CAAGGCTAAA
1840	1900	1960	2020	2080	2140	2200	2260	2320	2380
TTCCACGGGT	CTGTCCCCAC	CCAGGGGCTG	CTGCCCTGGG	GCCTCTGTAG	CTCGGGGGCA	GGGGCTC'PAG	TGGTTACGCG	TCTTCCCTTC	TCCCTTTAGG
AAGGTGCCCA	GACAGGGGTG	GGTCCCCGAC	GACGGGACCC	CGGAGACATC	GAGCCCCCGT	CCCCGAGAT'C	ACCAATGCGC	AGAAGGGAAG	AGGGAAATCC
1830	1890	1950	2010	2070	2130	2180 2190	2250	2310	2370
TGATGGTTCT	GCGGGTCCCA	TGGGGCTCAG	CCAGCAGCAC	CACAGCCCCT	TCCATGCCCA	TCAGGCGGAA AGAACCAGCT	GCGGGIGTGG	CCTTTCGCTT	AATCGGGGCA
ACTACCAAGA	CGCCCAGGGT	ACCCCGAGTC	GGTCGTCGTG	G'IGTCGGGGA	AGGTACGGGT	ACTCCGCCTT TCTTGGTCGA	CGCCCACACC	GGAAAGCGAA	TTAGCCCCGT
1820	1880	1940	2000	2060	2120	2180	2240	2300	2360
TGCGAGAC'IG	GGGAGGCAGA	CCCCCTAGGG	GGCCCTCCCT	GGGACAGACA	CCTCCCGACC	TGAGGCGGAA	ATTFAAGCGCG	AGCGCCCGCT	TCAAGCTCTA
ACGCTCTGAC	CCCTCCGTCT	GGGGGATCCC	CCGGGAGGGA	CCCTGTCTGT	GGAGGGCTGG	ACTCCGCCTT	TAATTCGCGC	TCGCGGGCGA	AGTTCGAGAT
1810	1870	1930	1990	2050	2110	2170	2230	2290	2350
CCTGGGCCCC	AGTGGCA1GA	TGTCCCTGGG	TTGCCAGCGT	GGAGCCCCTG	GCGCCCCTGT	C'FATGGC'ITC	GTAGCGGCGC	CCAGCGCCCT	GCTTTCCCCG
GGACCCGGGG	TCACCGTACT	ACACGGACCC	AACGGTCGCA	CCTCGGGGAC	CGCGGGGACA	GATACCGAAG	CATCGCCGCG	GGTCGCGGGA	CGAAAGGGGC

FIGURE 19D

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2460 CCATCGCCCT GGTAGCGGGA	2520 GGACTCTTGT CCTGAGAACA	2580 PPTTGATYTA TAAGGGATYY AAAACTAAAT ATTCCCTAAA	2640 AACGCGAATT TTGCGCTTAA	2700 CAGGCAGGCA GTCCGTCCGT	2760 CTAACTCCGC GATTGAGGCG	2820 GCCCCATGGC TGACTAATTT CGGGGTACCG ACTGATTAAA	2880 AAGTAGTGAG TYCATCACTC	2940 GCTGCGATTT CGACGCTAAA	3000 CCCGCTGCCA GGGCCACGGT
2450 ACGTAGTGGG TGCATCACCC	2510 CTTTAATAGT GAAATTATCA		2620 2630 AGCTGATTTA ACAAAAATTT TCGACTAAAT TGTTTTTAAA	2690 CCAGGCTCCC GGTCCGAGGG	2740 2750 CAGCAACCAT AGTCCCGCCC GTCGTTGGTA TCAGGGCGGG		2870 GCTATTCCAG CGATAAGGTC	2930 2940 ACAGCTCAGG GCTGCGATTT TGTCGAGTCC CGACGCTAAA	2990 GGATTTTATC CCTAAAATAG
2440 GTGATGGTTC CACTACCAAG	2500 AGTCCACGTT TCAGGTGCAA	2560 CGGTCTATTC GCCAGATAAG	2620 AGCTGATTTA TCGACTAAAT	2680 TGGAAAGTCC ACCTTTCAGG		2800 CCCATTCTCC GGGTAAGAGG	2860 CGGCCTCTGA GCCGGAGACT	2920 AAAAGCT'IGG TYYYTCGAACC	2980 AAGGCTGGTA TTCCGACCAY
2430 2440 CTTGATTAGG GTGATGGTTC GAACTAATCC CACTACCAAG	2480 2490 TTTTCGCCCT TTGACGTTGG AAAAGCGGGA AACTGCAACC	2550 2560 AACCCTATCT CGGTCTATTC TTGGGATAGA GCCAGATAAG	2610 TTAAAAAATG AATTTTTTAC	2670 AĞTTAGGGTG TCAATCCCAC	2730 CTCAATTAGT GAGTTAATCA	2790 CCCAGTYCCG GGGTCAAGGC	2850 2860 GAGGCCGCCT CGGCTCTGA CTCCGGCGGA GCCGGAGACT	GAGGCTTYTT TGGAGGCCTA GGCTTYTGCA CTCCGAAAAA ACCTCCGGAT CCGAAAACGT	TYGACGCCAA TCCTAGCGTG AAGGCTGGTA AACTGCCGTT AGGATCGCAC TYCCGACCAT
2420 CCCCAAAAA GGGGTTTTTT	2480 TTTTCGCCCT AAAAGCGGGA	2540 AACAACACTC TTGTTGTGAG	2610 GGCCTATTGG TTAAAAATG CCGGATAACC AATTTTTAC	2660 AATGTGTGTC TTACACACAG	2720 AAGCATGCAT TTCGTACGTA	2780 CCTAACTCCG GGATTGAGGC	2840 TGCAGAGGCC ACGTCTCCGG	2900 TGGAGGCCTA ACCTCCGGAT	CGCGCCAAAC TTGACGCAA TCCTAGCGTG AAGGCTGGTA GCGCGCII'I'I'I' AACTGCCGTI'I AGGATCGCAC TTCCGACCAT
2410 GGCACCTCGA CCGTGGAGCT	2470 GATAGACGGT CTATCTGCCA	2530 TCCAAAC'I'GG AGGTTT'GACC	2590 TGGGGATTTC ACCCCTAAAG	2650 AATTICTGTGG TTAAGACACC	2710 GAAGTATGCA CTTCATACGT	2770 CCATCCCGCC GGTAGGGCGG	2830 TTTTTATTYFA AAAATAAAT	2890 GAGGCTTTTT CTCCGAAAAA	2950 CGCGCCAAAC GCGCGGITIIC
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FIGURE 19E

3060	3120	3180	3240	3300	3360	3420	3480	3540	3600
ATTGGCAAGA	AGAATGACCA	ACCTGGTTCT	AGTAGAGAAC	GCCTTAAGAC	GGAGGCAGTT	ACAAGGATCA	TYTAAACTTC	AAGTATAAGT	GCTCCCCTCC
TAACCGTTCT	TCTTACTGGT	TGGACCAAGA	TCATCTCTTG	CGGAATTCTG	CCTCCGTCAA	TGITCCTAGT	ATATTTGAAG	I'I'CATATITCA	CGAGGGGAGG
3050	3110	3170	3230	3290	3340 3350	3410	3470	3510 3520 3530 CTCTCTGAGG TCCAGGAGGA AAAAGGCATC GAGAGACTCC AGGTCCTCCT TTTTCCGTAG	3590 3600
AAATATGGGG	GTACTTCCAA	GGGTAGGAAA	TATAGTTCTC	TTTGGATGAT	TAGACATGGT TTGGATAGTC	ACTCTFTGTG	TTTGGGGAAA TATAAACTTC		AAGATGCTTT CAAGTTCTCT GCTCCCCTCC
TYTATACCCC	CATGAAGGTT	CCCATCCTTT	ATATCAAGAG	AAACCTACTA	ATCTGTACCA AACCTATCAG	TGAGAAACAC	AAACCCCTTT ATATTTGAAG		TTCTACGAAA GTTCAAGAGA CGAGGGGAGG
3040 CCGTGTCCCA GGCACAGGGT	3100 ACGAGTTCAA TGCTCAAGTT	3160 TGGTGATFAT ACCACTAATA	3220 ACAGAATTAA TGTCTTAATT	3280 TTGCCAAAAG AACGGTTTTC		3400 GCCACCTTAG CGGTGGAATIC	3460 CAGAAATTGA GTCTTTAACT	3520 TCCAGGAGGA AGGTCC'ICCT	
3030	3090 3100	3150 3160	3210 3220	3270	3330	3390	3450		3550 3560 3570
TGCATCGTCG	CCGCTCAGGA ACGAGTTCAA	AAACAGAATC TGGTGATFAT	CCTTTAAAGG ACAGAATTAA	GCTCATTITC	GCAAGTAAAG	AATCAACCAG	ACGTTTTTCC		TTGAAGTCTA CGAGAAGAAA GACTAACAGG
ACGTAGCAGC	GGCGAGTCCT TGCTCAAGTT	TFTGTCTTAG ACCACTAATA	GGAAATTTCC TGTCTTAATT	CGAGTAAAAG	CGTTCATTTC	TTAGTTGGTC	TGCAAAAAGG		AACTTCAGAT GCTCTTCTTT CTGATTGTCC
3020		3140	3200	3260	3320	3380	3430	3500	3560
ACCATTGAAC		AGTGGAAGGT	GAAGAATCGA	ACCACGAGGA	TTATTGAACA ACCGGAATTG	GGAAGCCATG	TGCAGGAATT TGAAAGTGAC	CCCAGGCGTC	CGAGAAGAAA
TGGTAACTTG		TCACCTTCCA	CTTCTTAGCT	TGGTGCTCCT	AATAACTTGT TGGCCTTAAC	CCTTCGGTAC	ACGTCCTTAA ACTTTCACTG	GGGTCCGCAG	GCTCTTCTYTT
3020	3070	3130		3250	3310	3370	3430	3490	3550
TCATGGTTCG ACCATTGAAC	ACGGAGACCT	CAACCTCTTC		TCAAAGAACC	TTATTGAACA	CTG'FFFACCA	TGCAGGAATT	TCCCAGAATA	TTGAAGTCTA
AGTACCAAGC TGGTAACTTG	TGCCTCTGGA	GTTGGAGAAG		AGITTCTTGG	AATAACTTGT	GACAAA'FGGT	ACGTCCTTAA	AGGGTCYTAT	AACTTCAGAT

FIGURE 19F

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3660	3720	3780	3840	3900	3960	4020	4080	4140	4200
TCTTTGTGAA	TTTAAAGCTC	TAATTGTTTG	ATGCCTTTAA	CTACTGCTGA	AGGACTTTCC	TTGCTTGCTT	TGGAAAATA	TTTTTCTTAC	GTACCTTTAG
AGAAACACTT	AAATTTCGAG	ATTAACAAAC	TACGGAAATT	GATGACGACT	TCCTGAAAGG	AACGAACGAA	ACCTTTTTAT	AAAAAGAATG	CATGGAAATC
3650	3710	3770	3830	3890	3950	4010	4070	4130	4190
GCTTTAGATC	CCTACAGAGA	C'FACTGATTC	CAGTGGTGGA	GATGATGAGG	GAAGACCCCA	AATAGAACTC	AAGAAAATTA	AACATACTGT	CAAAAATTGT
CGAAATCTAG	GGATGTCTCT	GATGACTAAG	GTCACCACCT	CTACTACTCC	CTTCTGGGGT	TTATCTTGAG	TTCTTTTAAT	TYGTATGACA	GTTTTTAACA
3640	3700	3760	3820	3880	3940	4000	4060	4120	4180
ACTITITICETG	GGACAAACTA	ATGTGTTAAA	TGAATGGGAG	GCCATCTAGT	GAGAAAGGTA	TGTGTTTAGT	ACTGCTATAC	TTATAATCAT	TAACTATGCT
TGAAAACGAC	CCTGITTTGAT	TACACAATTT	ACTTACCCTC	CGGTAGATCA	CTCTTTCCAT	ACACAAATCA	TGACGATATG	AATATTAGTA	ATTGATACGA
3630	3690	3750	3810	3870	3930	3990	4050	4110	4170
AGACCATGGG	TGACATAATT	TAAGTGTATA	ATGGAACTGA	CAGAAGAAAT	CAAAAAAGAA	TGAGTCATGC	AAAAAGCTGC	GGCATAACAG	CTGCTATTAA
TCTGGTACCC	ACTGTATTAA	ATTCACATAT	TACCTTGACT	GTCTTCTTTA	GTTTTTTCTT	ACTCAGTACG	TTTTTCGACG	CCGTATTGTC	GACGATAATT
3620	3680	3740	3800	3860	3920	3990 3990	4040	4100	4160
CATTTTTATA	TTCTGTGGTG	ATAAAATITIT	ATTCCAACCT	CTGTTTTGCT	TCTACTCCTC	CTAAGTTTTT TGAGTCATGC	ACCACAAAGG	TTTATAAGTA	CATAGAGTGT
GTAAAAATAT	AAGACACCAC	TATITITAAAA	TAAGGTTGGA	GACAAAACGA	AGATGAGGAG	GATTCAAAAA ACTCAGTACG	TGGTGTTTCC	AAATATTCAT	GTATCTCACA
3610	3670	3730	3790	3850	3910	3970	4030	4090	4150
TAAAGCTATG	GGAACCTTAC	TAAGGTAAAT	TGTATTFFAG	TGAGGAAAAC	CTCTCAACAT	TYCAGAATTG	YGCTATTTAC	TTCTGTAACC	TCCACACAGG
ATTTCGATAC	CCTTGGAATG	ATTCCATTFA	ACATAAAATC	ACTCCTTTTG	GAGAGTTGTA	AAGTCTYAAC	ACGATAAATG	AAGACAT'IGG	AGGTGTGTCCC

TTAATAAGGA ATATTTGATG TATAGTGCCT TGACTAGAGA

GAAAAATTAA ACATTTCCCC AATTATTCCT TATAAACTAC ATATCACGGA ACTGATCTCT

TGTAAAGGGG

CTTTTTAATT

CCATACCACA TYTGTAGAGG TYTTACYTGC TYTAAAAAAC CTCCCACACC GACGGTGTCC CAATTGTTGT TGTTAACTTG TYTATTGCAG TCATCAATGT ATCTTATCAT GTCTGGATCG TRATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTC ACAAATAAAG ACTICCATTICT AGTIGINGST TIGTICCAAACT CATCAATGTA TICTTATCATG AGGGGGACTI' GGACTITIGTA TITITACTITAC GITITAACAACA ACAATIGAAC AAA'TAACGIC GAATATTACC AATGITTATT ICGITATCGI AGIGITTAAA GIGITTAITT CGIAAAAAA ATCAACACCA AACAGGTTTG AGTAGTTACA TAGAATAGTA CAGACCTAGC CGACCTACTA GGAGGTCGCG CCCCTAGAGT ACGACCTCAA GAAGCGGGTG GGGTTGAACA AATAACGTCG AATATTACCA AIGTTTAITT CGTTATCGTA GTGTTTAAAG TGTTTAITTC TUACGTAAGA TCAACACCAA ACAGGTTIGA GTAGITIACAT AGAATAGTAC GTCGACCTCT AGCTAGAGCT TGGCGTAATC ATGGTCATAG CTGTTTCCTG CCCAACTTGT TCGATCTCGA ACCGCATTAG TACCAGTATC GACAAAGGAC AAAATGAACG AAATTTTTTG GGGGATCTCA TGCTGGAGTT CTTCGCCCAC 4310 4490 4550 4670 4370 4610 4730 4300 4360 4540 4720 4480 4600 4660 AAACATCTCC CCTGAAACAT AAAATGAATG CACTGCATTC TAGTTGTGGT TTGTCCAAAC 4290 4530 4710 4470 4590 4650 4350 GGTATGGTGT CAGCTGGAGA CCTCCAGCGC 4340 4460 1580 4520 4640 4700 TCATAATCAG CATYFTYIYIY AGACATATGG GCTGGATGAT TCTGTATACC AGTATTAGTC CTTATAATGG GTGACGTAAG TTATTGCAGC TCCCCCTGAA 4390 4570 GTAAAAAAAG

FIGURE 19H

41150

TGTGAAATTG 1TATCCGCTC ACAATTCCAC ACAACATACG AGCCGGAAGC ATAAAGTGTA ACACTTTAAC AATAGGCGAG 1'GTTAAGGTG TGTTICTA1'GC 1'CGGCCTTCG TAI'ITCACAT

4770

4860	4920	4980	5040	5100	5160	5220	5280	5340	5400
TCACTGCCCG	CGCGCGGGGA	CTGCGCTCGG	TTATCCACAG	GCCAGGAACC	GAGCATCACA	TACCAGGCGT	ACCGGATACC	TGTAGGTATC	CCCGTTCAGC
AGTGACGGGC	GCGCGCCCT	GACGCGAGCC	AATAGGTGTC	CGGTCCTTGG	CTCGTAGTGT	ATGGTCCGCA	TGGCCTATGG	ACATCCATAG	GGGCAAGTCG
4850	4910	4960 4970	5030	5090	5150	5210	5270 5280	5320 5330	5380 5380
TGCGTTGCGC	AATCGGCCAA	CTTCCTCGCT CACTGACTCG	GGTAATACGG	CCAGCAAAAG	CCCCCCTGAC	ACTATAAAGA	CCTGCCGCTT ACCGGATACC	CGCTPTCTCA ATGCTCACGC	TGGGCTGTGT GCACGAACCC
ACGCAACGCG	TTAGCCGGTT	GAAGGAGCGA GTGACTGAGC	CCATTATGCC	GGTCGTTTTC	GGGGGGACTG	TGATATTTCT	GGACGGCGAA TGGCCTATGG	GCGAAAGAGT TACGAGTGCG	ACCCGACACA CGTGCTTGGG
4840 TCACATTAAT AGTGTAATTA	4890 TCGTGCCAGC TGCATTAATG AGCACGGTCG ACGTAATTAC		5020 ACTCAAAGGC TGAGTTTCCG	5080 GAGCAAAAGG CTCGITTTTCC	5140 ATAGGCTCCG TATCCGAGGC	5190 5200 AGGTGGCGAA ACCCGACAGG TCCACCGCTT TGGGCTGTCC	5260 CTGTTCCGAC GACAAGGCTG		5380 TGGGCTGTGT ACCCGACACA
4830	4890	4950	5010	5070	5130	5190	5250	5310	5370
GYGAGCTAAC	TCGTGCCAGC	CGCTCTPTCCG	GTATCAGCTC	AAGAACATGT	GCGTTTTTCC	AGGTGGCGAA	GTGCGCTCTC	GGAAGCGTGG	CGCTCCAAGC
CACTCGATTG	AGCACGGTCG	GCGAGAAGGC	CATAGTCGAG	TTCTTGTACA	CGCAAAAAGG	TCCACCGCTT	CACGCGAGAG	CCTTCGCACC	GCGAGGTTCG
4820	4880	4940	5000	5060	5120	5180	5240	5290 5300	5360
TGCCTAATGA	GGGAAACCTG	GCGTATTGGG	GCGGCGAGCG	TAACGCAGGA	CGCGTTGCTG	CTCAAGTCAG	AAGCTCCCTC	TGTCCGCCTT TCTCCCTTCG	GTAGGTCGTT
ACGGATTACT	CCCTTTGGAC	CGCATAACCC	CGCCGCTCGC	ATTGCGTCCT	GCGCAACGAC	GAGTTCAGTC	TTCGAGGGAG	ACAGGCGGAA AGAGGGAAGC	CATCCAGCAA
4810	4870	4930	4990	5050	5110	5170	5230	5290	5350
AAGCC'IGGGG	CTTTCCAGTC	GAGGCGGTFF	TCGTTCGGCT	AATCAGGGGA	GTAAAAAGGC	AAAATCGACG	TTCCCCCTGG	TGTCCGCCTT	TCAGTTCGGT
T'I'CGGACCCC	GAAAGGTCAG	CTCCGCCAAA	AGCAAGCCGA	TTAGTCCCCT	CATTTTTCCG	1TTTPAGCTGC	AAGGGGGACC	ACAGGCGGAA	AGTCAAGCCA

5460	5520	5580	5640	5690 5700	5760	5820	5880	5940	6000
AGACACGACT	GTAGGCGGTG	GTATTTGGTA	TGATCCGGCA	GCAGCAGATT ACGCGCAGAA	CAGTGGAACG	ACCTAGATCC	ACTTGGTCTG	TTTCGTTCAT	TTACCATCTG
TCTGTGCTGA	CATCCGCCAC	CATAAACCAT	ACTAGGCCGT	CGTCGTCTAA TGCGCGTCTT	GTCACCTTGC	TGGATCTAGG	TGAACCAGAC	AAAGCAAGTA	AATGGTAGAC
5460 GTCTTGAGTC CAACCCGGTA AGACACGACT CAGAACTCAG GTTGGGCCAT TCTGTGCTGA	ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG TGACCATTGT CCTAATCGTC TCGCTCCATA CATCCGCCAC	5570 TAGAAGGACA ATCTTCCTGT	5630 5640 TGGTAGCTCT TGATCCGGCA ACCATCGAGA ACTAGGCCGT	5690 5700 GCAGCAGATT ACGCGCAGAA CGTCGTCTAA TGCGCGTCTT	5750 GTCTGACGCT CAGACTGCGA	5810 AAGGATCTTC TTCCTAGAAG	5870 ATATGAGTAA TATACTCATT	5930 GATCTGTCTA CTAGACAGAT	5990 ACGGGAGGC TGCCCTCCCG
5440	5500	5560	5620	5680	5740	5800	5860	5920	5970 5980
GTCTTGAGTC	GGATTAGCAG	ACGGCTACAC	GAAAAAGAGT	TTGTTTGCAA	TTTCTACGGG	GATTATCAAA	TCTAAAGTAT	CTATCTCAGC	GTCGTGTAGA TAACTACGAT
CAGAACTCAG	CCTAATCGTC	TGCCGATGTG	CTTTTTCTCA	AACAAACGTT	AAAGATGCCC	CTAATAGITIT	AGATTTCATA	GATAGAGTCG	CAGCACATCT ATTGATGCTA
5430	5490	5550	5610	5670	5730	5790	5850	5910	
GGTAACTATC	ACTGGTAACA	TGGCCTAACT	GTTACCTTCG	GGTGGTTTTT	CCTTTGATCT	TTGGTCATGA	TTTAAATCAA	AGTGAGGCAC	
CCATTGATAG	TGACCATTGT	ACCGGATTGA	CAATGGAAGC	CCACCAAAAA	GGAAACTAGA	AACCAGTACT	AAATTTAGTT	TCACTCCGTG	
5420	5480	5540	5600	5660	5720	5780	5840	5900	5950 5950 CCATACTICCCC
CGCCTTATCC	GGCAGCAGCC	CTTGAAGTGG	GCTGAAGCCA	CGCTGGTAGC	TCAAGAAGAT	TTAAGGGATT	AAAATGAAGT	ATGCTTAATC	
GCGGAATAGG	CCGTCGTCGG	GAACTTCACC	CGACTTCGGT	GCGACCATCG	AGTTCTTCTA	AATTCCCTAA	TTTTACTTCA	TACGAATTAG	
5410	5470	5530	5590	5650	5710	5770	5830	5890	5950
CCGACCGCTG	TATCGCCACT	C'TACAGAGTT	TCTGCGCTCT	AACAAACCAC	AAAAAGGATC	AAAACTCACG	TYFTAAATTA	ACAGTTACCA	CCATAGITYGC
GGCTGGCGAC	ATAGCGGTGA	GATG'FC'FCAA	AGACGCGAGA	TTGTTTGGTG	T'FTTICCTAG	TYTTGAGTGC	AAAATTFAAT	TGTCAATGGT	GGTATYNACG
							421-		

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6060 TTATCAGCAA AATAGTCGTT 6120 TCCGCCTCCA AGGCGGAGGT	6180 AATAGTTTGC TTATCAAACG 6240	CCATACCGAA 6300 TTGTGCAAAA AACACGTTTTT	6360 GCAGTGTTAT CGTCACAATA	6420 GTAAGATGCT CATTCTACGA	6480 CGGCGACCGA GCCGCTGGCT	6540 ACTTTAAAAG TGAAATTTTC	6600 CCGCTGTTGA GCCGACAACT
6050 GGCTCCAGAT CCGAGGTCTA 6110 TGCAACTTTA	6170 TYCGCCAGTT AAGCGGTCAA 6230	CICGLOSTIT GAGCAGCAAA ATCCCCCATG TAGGGGGTAC	6350 TAAGTTGGCC ATTCAACCGG	6410 CATGCCATCC GTACGGTAGG	6470 ATAGIGTATG TATCACATAC	6530 ACATAGCAGA TGTATCGTCT	6590 AAGGATCTTA TICCTAGAAT
6040 CACGCTCACC GTGCGAGTGG 6100 GAAGTGGTCC CTTTCACCAGG	6160 GAGTAAGTAG CTCATTCATC	TGGTGTCACG ACCACAGTGC 6280 GAGTTACATG CTCAATGTAC	6340 TTGTCAGAAG AACAGTCTTC	6400 CTCTTACTGT GAGAATGACA	6460 CATTCTGAGA GTAAGACTCT	6520 ATACCGCGCC TATGGCGCGG	6580 GAAAACTCTC C'I''I''TGAGAG
6030 GCCGCGAGACC GGCGCTCTGG 6090 GCCGAGCGCA	6150 CGGGAAGCTA GCCCTTCGAT	ACAGGCATCG TGTCCGTAGC 6270 CGATCAAGGC GCTAGTTCCG	6330 CCTCCGATCG GGAGGCTAGC	6390 CTGCATAATT GACGTATTAA	6450 TCAACCAAGT AGTTGGTTCA	6510 ATACGGGATA TATGCCCTAT	6560 6570 TGGAAAACGT TCTTCGGGGC ACCTYTTGCA AGAAGCCCCG
6020 TGCAATGATA ACGTTACTAT 6080 AGCCGGAAGG	6140 TAATTGTTGC ATTAACAACG	TGCCATTGCT ACGGTAACGA 6260 CGGTTCCCAA GCCAAGGGTT	6320 CTCCTTCGGT GAGGAAGCCA	6380 TATGGCAGCA ATACCGTCGT	6440 TGGTGAGTAC ACCACTCATG	6500 CCCGGCGTCA GGGCCGCAGT	
6010 GCCCCAGINGC CGGGGICACG 6070 TAAACCAGCC	6130 TCCAGTCTAT AGGTCAGATA	GCAACGTTGT CGTTGCAACA CGTTGCAGCA CATTCAGCTC GTAAGTCGAG	6310 AAGCGGTTAG TYCGCCAATC	6370 CACTCATGGT GTGAGTACCA	6430 1TTCTCTGAGC AAAGACACTG	6490 GTTGCTC'TTG CAACGAGAAC	6550 TGCTCATCAT ACGAGTAGTA

FIGURE 19K

6660	6720	6780	6840	6900	6960	7020	7080	7140	7200
TTTACTTTCA	GGAATAAGGG	AGCATTTATC	AAACAAATAG	GGAGATCTGC	TTAATTTTAT	GGTCGACTCT	CTTGTGTGTT	GCTTGACCGA	ATCTACGGGC
AAATGAAAGT	CCTTATTCCC	TCGTAAATAG	TTTGTTTATC	CCTCTAGACG	AATTAAAATA	CCAGCTGAGA	GAACACACAA	CGAACTGGCT	TACATGCCCG
6650	6710	6770	6830	6890	6950	7010	7070	7130	7190
TYCAGCATCT	CGCAAAAAG	ATATTATTGA	TTAGAAAAAT	CGACGGATCG	ACCTTTTTTT	GATCCCCTAT	CTGCTCCCTG	ACAAGGCAAG	CTGCTTCGCG
AAGTCGTAGA	GCGTTTTTTC	TATAATAACT	AATCTTTTTA	GCTGCCTAGC	TGGAAAAAA	CTAGGGGATA	GACGAGGGAC	TGTTCCGTTC	GACGAAGCGC
6640	6700	6760	6820	6880	6940	7000	7060	7120	7180
CCAACTGATC	GGCAAAATGC	TCCTTTTTCA	TTGAATGTAT	CACCTGACGT	AGCCAGAGTA	CCGATCTCCC	AAGCCAGTAT	TTAAGCTACA	GCGTTTTTGCG
GGTTGACTAG	CCGTTYTACG	AGGAAAAAGT	AACTTACATA	GTGGACTGCA	TCGGTCTCAT	GGCTAGAGGG	TTCGGTCATA	AATTCGATGT	CGCAAAACGC
6630	6690	6750	6810	6870	6930	6990	7050	7110	7170
ACTCGTGCAC	AAAACAGGAA	CTCATACTCT	GGATACATAT	CGAAAAGTGC	GGCTTCGAAT	GAGTTTGGCG	CCGCATAGTT	CGAGCAAAAT	TTAGGGTTAG
TGAGCACGTG	TYTYGYCCTY	GAGTATGAGA	CCTATGTATA	GCTTTTCACG	CCGAAGCTTA	CTCAAACCGC	GGCGTATCAA	GCTCGTTTTTA	AATCCCAATC
6620	6680	6740	6800	6860	6920	6980	7040	7100	7160
GATGTAACCC	TGGGTGAGCA	ATGTTGAATA	TCTCATGAGC	CACATTTCCC	GAGGCGCGCC	TTTTGAGATG	TGCTCTGATG	GAGTAGTGCG	AAGAATCTGC
CTACATTGGG	ACCCACTCGT	TACAACTTAT	AGAGTACTCG	GTGTAAAGGG	CTCCGCGCGG	AAAACTCTAC	ACGAGACTAC	CTCATCACGC	TrctTAGACG
6610 GATCCAGTTC CTAGGTCAAG	6670 CCAGCGITITC GGTCGCAAAG	6730 CGACACGGAA GCTGTGCCTT	6790 AGGGTTATTG TCCCAATAAC	6850 GGGTTCCGCG CCCAAGGCGC	6910 TAGGTGACCT ATCCACTGGA	6970 TTTATTTTT	7030 CAGTACAATC GTCATGTTAG	7090 GGAGGTCGCT CCTCCAGCGA	7150 CAATIGCATG GTTAACGTAC

						•		
7260 TTACGGGGTC AATGCCCCAG 7320 ATGGCCCGCC	TACCGGGCGG 7380 TYCCCATAGT AAGGGTATCA	7440 AAACTGCCCA TTTGACGGGT	7500 TCAATGACGG AGTTACTGCC	7560 CTACTYGGCA GATGAACCGT	7620 AGTACATCAA TCATGTAGTT	7680 TTGACGTCAA AACTGCAGTT	7740 ACAACTCCGC TGTTGAGGCG	7800 GCAGAGCTCT CGTCTCGAGA
7250 TAGTAATCAA A1CATTAGTT 7310 CTTACGGTAA	GAATGCCATT 7370 ATGACGTATG TACTGCATAC	7430 TATTTACGGT ATAAATGCCA	7490 CCTATTGACG GGATAACTGC	7550 TGGGACTTTC ACCCTGAAAG	7610 CGGTTTTYGGC GCCAAAACCG	7670 CTCCACCCCA GAGGTGGGGT	7730 AAATGTCGTA TTTACAGCAT	7790 GTCTATATAA CAGATATATT
7240 TAGTFATTAA ATCAATAATT 7300 CGTTACATAA	GCAATGTATT 7360 GACGTCAATA CTGCAG'ITAT	7420 ATGGGTGGAC TACCCACCTG	7480 AAGTACGCCC TTCATGCGGG	7540 CATGACCTTA GTACTGGAAT	7600 CATGGTGATG GTACCACTAC	7660 ATTTCCAAGT TAAAGGTTCA	7720 GGACTTTCCA CCTGAAAGGT	7780 ACGGTGGGAG TGCCACCCFC
7230 GATTATTGAC CTAATAACTG 7290 TGGAGTTCCG	ACCTCAAGGC 7350 CCCGCCCATT GGGCGGGTAA	7410 ATTGACGTCA TAACTGCAGT	7470 ATCATATGCC TAGTATACGG	7530 ATGCCCAGTA TACGGGTCAT	7590 TCGCTATTAC AGCGATAATG	7650 ACTCACGGGG TGAGTGCCCC	7710 AAAATCAACG TTTTAGTTGC	7770 GTAGGCGTGT CATCCGCACA
7220 CGTTGACATT GCAACTGTAA 7280 AGCCCATATA	TCGGGTATAT 7340 CCCAACGACC GGGTTGCTCG	7400 GGGACTTTCC CCCTGAAAGG	7460 CATCAAGTGT GTAGTTCACA	7520 GCCTGGCATT CGGACCGTAA	7580 GTATTAGTCA CATAATCAGT	7640 TAGCGGTTTG ATCGCCAAAC	7700 TYTTGGCACC AAAACCGTGG	7760 CAAATGGGCG GTTPACCCGC
7210 CAGATATACG GTCTATATGC 7270 ATTAGTTCAT	TAATCAAGTA 7330 TGGCTGACCG ACCGACTGGC	7390 AACGCCAATA TTGCGGTTTA'F	7450 CTTGGCAGTA GAACCGTCAT	7510 TAAATGGCCC ATTTACCGGG	7570 GTACATCTAC CATGTAGATG	7630 TGGGCGTGGA ACCCGCACC'F	7690 TGGGAGTTTG ACCCTCAAAC	7750 CCCATTGACG GGGTAACTGC

FIGURE 19M

7810 7850 7860 CTGGCTAACT AGAGAACCCA CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GACCGATTGA TCTCTTGGGT GACGAATGAC CGAATAGCTT TAATTATGCT GAGTGATATC

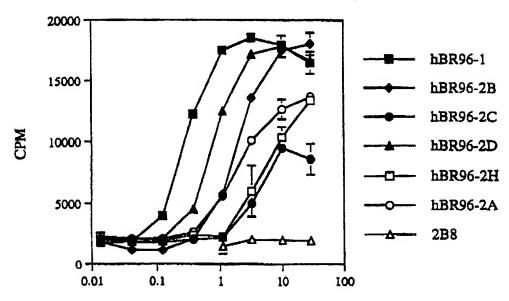
7880

7870 GGACACCCAA GCTT CCTCTGGGTT CGAA

FIGURE 19N

FIGURE 20

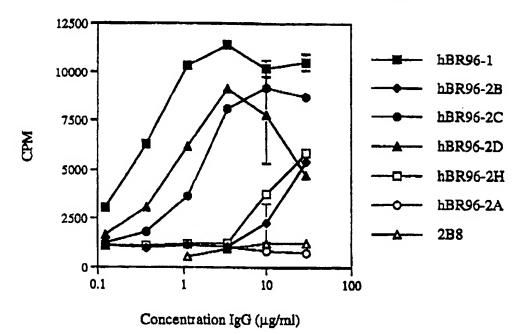
Complement Dependent Cytotoxicity



Concentration IgG (µg/ml)

FIGURE 21

Antibody Dependent Cell-Mediated Cytotoxicity



Binding activity of hBR96-2 constant region mutants on LeY-HSA

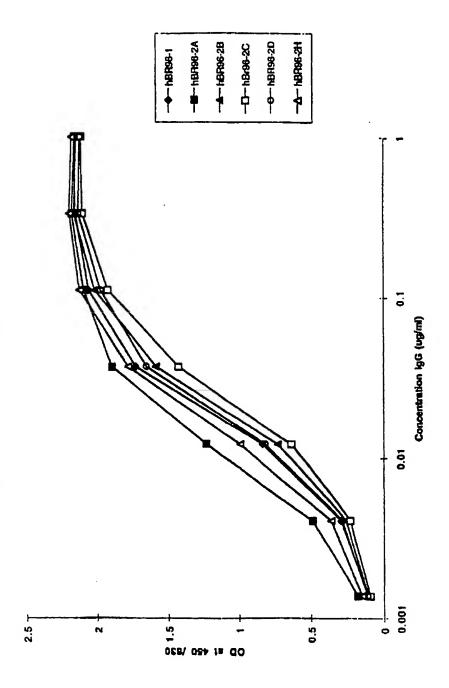
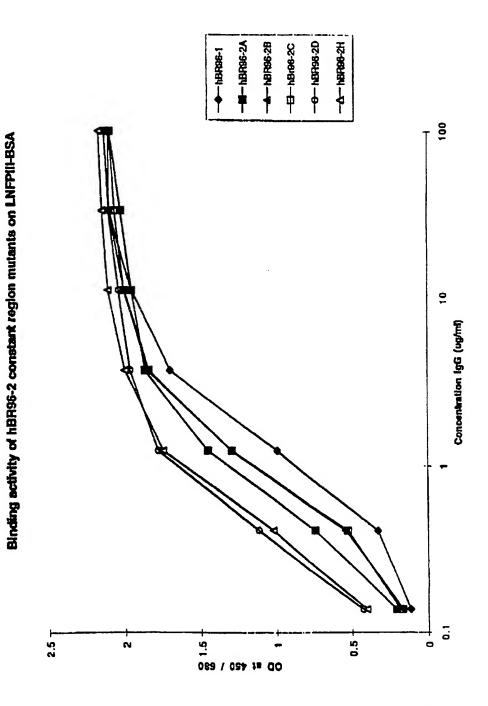


FIGURE 22

FIGURE 23



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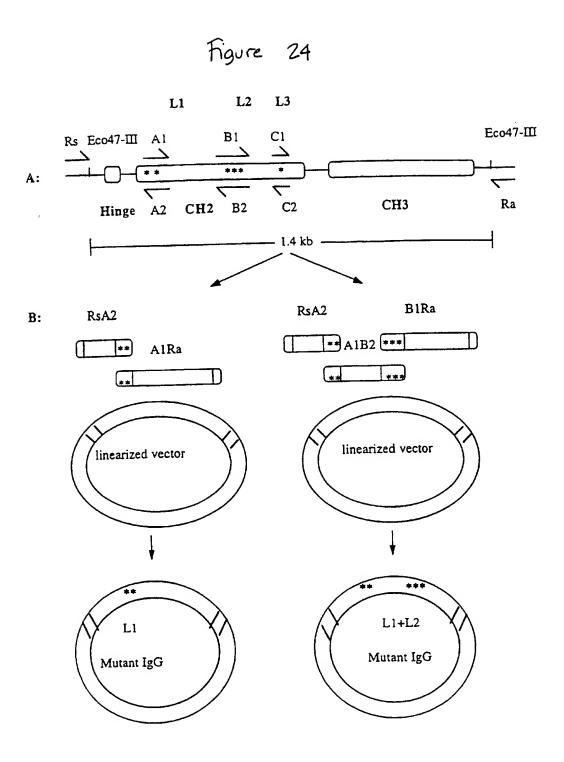


Figure 25

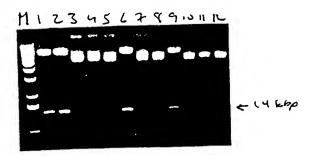


Figure 26

hBR96-2 Heavy Chain Variable Region (VH)

human IgGI constant

YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLSTQTY
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CHAPELOGOP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
18 120 321
TYRVVSVLTV LHQDWLNGKE YEUGVSNKAL PAPLEKTISK AKCOPREPQV
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

Figure 27

hBR96-2A: Heavy Chain Variable Region (VH)

EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY

51 61 71 81 91
ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL

101 111
"ADGAWFAYWG QGTLVTVSS

hBR96-2A: Human Heavy Chain IgG1 Constant Region ACH2

A STKGPSVFPL APSSKSTSCC ȚAALGCLVKO YFPEPVTVSW NSGALTSGVH

TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK

SCDKTHTCPP CP GQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA

VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM

HEALHNHYTG KSLSLSPGK

Figure 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

1 EVNLVESGGG LVQPGGSLKV SCVTSGFTFS DYYMYWVRQT PEKRLEWVAY
51 ISQGGDITDY PDTVKGRFTI SRDNAKNTLY LQMSRLKSED TAMYYCARGL
101 DDGAWFAYWG QGTLVTVSVA STKGPSVFPL APSSKSTSGG TAALGCLVKD
151 YPPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
201 ICMVMHKPSN TKVDKKVEPK SCDKTHTCPP CEGQPREPQV YTLPPSRDEL
251 TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS
301 KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

INTERNATIONAL SEARCH REPORT

Interna al Application No PCT/US 97/13562

a. classification of subject matter IPC 6 C12N15/62 A61 A61K47/48 A61K51/10 A61K38/17 A61K39/395 C12N1/21 C12N15/13 C07K16/00 C07K16/46 C07K16/30 //C07K19/00 C12N5/10 According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 $\,$ C07K $\,$ A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages S. GILLIES ET AL.: "Antigen binding and 1-8. X biological activities of engineered mutant 23-25 chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document -/--Patent family members are listed in annex. Further documents are listed in the continuation of box C. l XI To later document published after the international filing date or priority date and not in conflict with the application but * Special categories of cited documents : cited to understand the principle or theory underlying the *A* document defining the general state of the art which is not considered to be of particular relevance "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *E* earlier document but published on or after the international fiting date "L" document which may throw doubts on priority claim(s) or which is offed to establish the publication date of another citation or other special reason (as specified) "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu-"O" document referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled other means document published prior to the international filing date but *& document member of the same patent family later than the priority date claimed Date of mailing of the international search report Date of the actual completion of the international search 2 1. 01. 98 17 December 1997 **Authorized** officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2

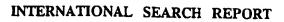
Form PCT/ISA/210 (second sheet) (July 1992)

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Fax: (+31-70) 340-3016

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Nooij, F



Intern nat Application No PCT/US 97/13562

C.(Continu	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/US 9//13562
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A		1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for Clq on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7,
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A	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7,
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INTERNATIONAL SEARCH REPORT

Intern al Application No PCT/US 97/13562

	tion) DOCUMENTS CONSIDERED TO BE RELEVANT	Relevant to claim No.
ategory *	Criation of document, with indication, where appropriate, of the relevant passages	Helevant to dialim No.
	L. TAN ET AL.: "Influence of the hinge region on complement activation, Clq binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document	1-8
	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application see examples	11-18, 23,25, 28,29, 31-52
	see claims	

Farm PCT/ISA/210 (continuation of second sheet) (July 1992)

International application No. PCT/US 97/13562

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)	
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:	
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: see FURTHER INFORMATION sheet PCT/ISA/210	
Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically: see FURTHER INFORMATION sheet PCT/ISA/210	
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).	
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)	
This International Searching Authority found multiple inventions in this international application, as follows:	
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.	
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.	
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:	
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:	
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.	

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark: Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr nal Application No PCT/US 97/13562

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 699756	A	06-03-96	AU 2834995 A CA 2155397 A JP 8191692 A	15-02-96 05-02-96 30-07-96

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